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Metabolomic Analysis of Drought Response in Guar (*Cyamopsis tetragonoloba* (L.) Taub.) Seeds

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Abstract

Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a legume crop valued for the gum (carbohydrate galactomannan) found in its seeds, which is widely used in the oil and mining industries. In this study, we assessed changes in seed metabolite content caused by drought stress in three guar genotypes (tolerant, neutral, and sensitive to drought) in order to gain an insight into molecular mechanisms of guar tolerance to drought. The most intense response to drought was observed in seeds of the tolerant genotype. In response to drought, the content of sugars and their derivatives in the seeds of all three genotypes changed: monosaccharide content increased and glycoside content decreased. However, the tolerant genotype accumulated a specific set of sugars and their derivatives, including galactinol, and demonstrated higher levels of tocopherols. The neutral genotype was characterized by higher content of glycosides and pentoses, while the sensitive genotype had higher accumulation of some specific sugars and derivatives, major phytosterols, and unsaturated C18 fatty acids. Overall, the accumulation of galactinol, phytol, and alpha-tocopherol in seeds was associated with guar drought tolerance. This finding expands the understanding of the molecular mechanisms of drought tolerance in guar and paves the way for breeding drought-tolerant guar varieties.

Keywords: guar; *Cyamopsis tetragonoloba*; metabolomics; drought

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1. Introduction

Guar (*Cyamopsis tetragonoloba* (L.) Taub., Fabaceae) is an annual, self-pollinated legume crop with multi-purpose applications, attracting significant interest from various industries and scientists in recent decades. This interest stems from the presence of the gum in its seeds—a water-soluble, nontoxic carbohydrate and hydrocolloid, used as a natural thickener, stabilizer, and emulsifier in food, textile, paper, cosmetics, medicine, and explosives production. However, the strategic importance of guar gum lies in its use in the oil (shale energy production or hydraulic fracturing) and mining industries [1]. As a drilling mud additive for deep horizontal drilling, guar gum offers unparalleled properties, and is cost-effective compared to other thickeners, including those obtained from plants. Another advantage of guar gum is that it is an environmentally friendly alternative to undesirable chemicals that can seep into groundwater aquifers, polluting water. The global guar gum market is currently valued at $13.4 billion, with an anticipated annual growth of 4.8% [2].

South Asia is the guar center of origin and primary production area. India and Pakistan dominate guar production and supply (80% and 15% of global output, respectively). The discovery of guar gum's importance triggered its introduction to various countries, where it is produced in limited quantities, including the USA, Australia, Southern Europe, South Africa, Sudan, Bangladesh, Brazil, Myanmar, Sri Lanka, China, Thailand, and the Russian Federation [3]. The crop is relatively unpretentious, thriving in infertile soils and semi-arid conditions [4,5]. While considered drought-tolerant, lack of water negatively impacts its morphology and productivity, particularly during pre-sowing and seed maturation [6]. Water supply is a major determinant of stable guar seed productivity [7]. Drought negatively affects phenotypic traits: plant height, leaf area, seed productivity components [8–11], and plant symbiotic apparatus [12–14]. The global guar gene pool demonstrates a genotype-specific response to water supply, including drought-tolerant and susceptible genotypes [11,15–18]. A similar differentiation was found in the guar VIR collection [7].

Optimal guar cultivation in new environments requires identifying *C. tetragonoloba* genotypes adapted to local conditions, particularly water scarcity. This necessitates understanding the physiological mechanisms of drought resistance and the underlying molecular–genetic basis of this trait. Unfortunately, such research on guar is limited, as are genomic and transcriptomic resources, and genetic resources remain incompletely characterized [19].

Enhancing cultivated plant productivity and uncovering plant–environment interactions are priority objectives in plant biology [20]. Metabolite profiling has been successfully employed to reveal systematic adaptation mechanisms to environmental stressors and identify stress biomarkers [21–25]. The implementation of high-throughput metabolite profiling has established the metabolome as an integral component of plant phenotypic descriptions, allowing genotypes to be differentiated more precisely [26–28]. This, in turn, enables a deeper understanding of genotype–phenotype relationships, facilitating the screening, selection, and development of cultivated crop varieties that are more adaptable and high-yielding [29–33].

Drought causes massive global crop yield losses [34]. The search for and development of resistant varieties remain crucial for combating this stressor, but such efforts must be grounded in understanding resistance mechanisms. This is where metabolomics can be valuable [35,36]. Substantial metabolomic profiling data has been accumulated for various crops, including rice, maize, wheat, barley, tomato, soybean, mung bean, pea, alfalfa, and cabbage [37]. However, only one study has investigated the metabolomic profiles of guar plants under drought stress [38]. Seed metabolomics is now emerging as a distinct field. This involves studying cultivar-specific patterns of metabolite accumulation in seeds [39–42]. The study of stress effects on seeds, including those of legumes, is also developing [43].

Metabolomic profiling generates large datasets, requiring appropriate analytical tools for interpretation. Metabolomic analysis aims to reduce data dimensionality, cluster, classify, and identify diagnostic features, such as biomarkers. The use of metabolomic profiling methods in combination with multivariate statistics and machine learning allows us to move from holistic data to the identification of biomarkers of specific processes occurring in the organism [44–46].

Principal component analysis (PCA), an unsupervised learning method, is the most common method of sample ordination. It identifies a small number of variables (principal components, PC) that preserve the variance in measured parameters. PCA enables the comparison of profiles as a whole [47]. Using metabolite content as a distance metric may not always be ideal due to dependencies on normalization. Therefore, a combination of non-parametric metrics and multidimensional scaling (MDS) could produce a more

appropriate and robust result [48]. This approach reduces the impact of normalization methods and has proven itself well in plant metabolomics studies [49].

One of the goals of the analysis is to identify condition-specific biomarkers. However, data can be noisy, especially in field experiments. Support vector machine (SVM), a supervised machine learning classification method, is resistant to noise. SVM has proven effective in identifying biomarkers in untargeted metabolomic profiling, even with small sample sizes and high variance [50,51]. Random forest (RF), another widely used classification method, is well-suited for handling data with numerous objects and features, including missing values; it is also robust to noise and overfitting [52].

Although guar is grown primarily for its seeds, there is currently a lack of knowledge about the molecular signatures of drought stress in guar seeds, as well as about biomarkers that could distinguish seeds of drought-tolerant and drought-sensitive guar varieties. This study aims to fill this gap and analyzes the metabolic features of guar seeds associated with their response to drought stress, which varies among accessions from the VIR collection. Here, GC-MS was used to analyze seed metabolite profiles of these three guar varieties under watering and drought conditions. PCA was used to demonstrate the effect of drought and the varietal specificity of metabolite profiles. RF and SVM methods were used to identify metabolites associated with drought and drought resistance. For the first time, this study demonstrates that various sugars accumulate in the seeds of all three guar genotypes in response to drought. Furthermore, galactinol, tocopherol, and phytol accumulate in the seeds of the tolerant guar genotype under drought stress conditions. The presence of these biomarkers may indicate accelerated seed ripening in this genotype as a strategy for responding to drought stress.

2. Materials and Methods

2.1. Plant Material

Three guar accessions from the VIR collection were studied. The genotypes were selected from a sample of 30 accessions that were phenotyped in the field in 2023–2024 near Krasnoslobodsk, Volgograd region, according to the methods adopted by VIR [7].

The studied samples were created according to the principle of maximum representativeness of the crop gene pool. Irrigated conditions and the induction of artificial drought were applied.

After harvesting, the elements of plants seed productivity (plant height, number of branches, pods, seed mass/plant, and others) were analyzed on 7–8 plants per genotype. The seed productivity of plants was accepted as the main criterion of adaptability to the irrigation regime. Based on this, differentiation of the studied sample into three groups was revealed: genotypes with maximum seed productivity under drought conditions in both years were considered drought-tolerant; those with maximum seed productivity under watering and minimum under drought were considered sensitive; and those forming approximately the same productivity under both regimes were considered neutral with respect to water supply. The attitude to drought remained unchanged in the majority of accessions in both years of the study.

One genotype from each group was selected for analysis: contrasting with drought and indifferent to it (neutral) (Table 1).

Table 1. Genotypes description.

Accession Name	Origin	VIR Number	Attitude to Drought
J.C.3118	India	k-52938	Tolerant
Local	India	k-52924	Sensitive
Suvti	India	k-52891	Neutral

2.2. Phenotyping

The climate of Krasnoslobodsk (48°42′ N, 44°34′ E) is temperate continental, with moderately cold winters and hot summers. The soils are chernozems with alluvial loams.

The genotypes were evaluated in the field under controlled watering, beneath a polyethylene canopy, to avoid natural rainfalls. In total, 30 guar accessions from the VIR collection were grown. Sowing was carried out on 28 April 2023 and on 18 April 2024. The experimental field was divided into two equal parts: control (watering (W)) and experiment (artificial drought (D)). The same set of accessions was sown on each half. Watering of the entire field continued until the beginning of fruit formation—the formation of the last bean in the lower cluster. In 2023, it occurred on 28 June, and in 2024, on 17 June. After that, watering was stopped on the experimental part of the field (D) in order to create artificial drought and was not resumed. On the control half of the field (W), watering was carried out in the same mode until harvesting. The spot watering rate was 5.0 L/plant per week in 2023 and 2.5 L/plant per week in 2024. The water regime of 2023 proved to be excessive for most accessions, while the one half lower in 2024 appeared favorable [7].

For metabolomic analysis, material of 2024 was used. Young beans (pods) from the first cluster (first inflorescence) of the plants were collected 6 weeks after the drought induction, on 29 July. By that time, plants under drought showed signs of wilting, but the pods, and especially the seeds, showed no distinctive features. The pods were shelled, and the seeds were frozen.

2.3. Extraction

Frozen guar seeds were preliminarily freeze-dried. Then, the dried seeds were ground with a pestle in a mortar. Metabolites were extracted from lyophilized guar seeds using methanol on a Vortex Genius 3 orbital shaker (IKA, Staufen im Breisgau, Germany). The extract was evaporated in a CentriVap Concentrator rotary evaporator (Labconco, Kansas City, MO, USA).

2.4. Sample Preparation

A silylating agent BSTFA:TMCS 99:1 (N,O-BIS (trimethylsilyltrifluoroacetamide with trimethylchlorosilane) and an internal standard (a solution of tricosane in pyridine 1 μg/μL) were added to the dry samples. Then, the samples were heated for 15 min at 100 °C on a Digi-Block (LabTech, Sorisole, Italy).

2.5. Gas Chromatography

Prepared samples were analyzed on an Agilent 6850 gas chromatograph with an Agilent 5977B quadrupole mass-selective detector (Agilent Technologies, Santa Clara, CA, USA). Samples were analyzed with Agilent 6850 chromatograph equipped HP-5ms Ultra Inert (Agilent Technologies, Santa Clara, CA, USA) capillary column and coupled with an Agilent 5977B quadrupole mass selective detector (Agilent Technologies, Santa Clara, CA, USA). The flow rate of helium was 1.3 mL/min. Column temperature regime: initial—60 °C, final—325 °C, rate—6 °C per min. The inlet was operated in splitless mode at the temperature of 250 °C. Electron impact ionization was performed at 70 V and an ion source temperature of 230 °C. The mass range of detected ions was 50–800.

2.6. GC-MS Data Processing

The GC-MS data analysis was processed using the PARADISe 6.01 software (Department of Food Science, Faculty of Science, University of Copenhagen, Denmark) [53], coupled with NIST MS Search 2.4 (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA). In addition, for deconvolution and metabolite identification, the

AMDIS 2.71 (Automated Mass Spectral Deconvolution and Identification System, NIST, Gaithersburg, MD, USA) was used. Analytes were identified by mass-spectra and Kovats retention indices using the libraries: NIST2020, Golm Metabolome Database (GMD) [54]. An "in-house" library created by the Laboratory of Analytical Phytochemistry, was also used (BIN RAS assignment # 124020100140-7).

2.7. Statistical Analysis and Visualization

Data analysis was performed in the R 4.3.1 "Beagle Scouts" environment (R Core Team, 2023). A total of 8–10 biological replicates from three experimental fields were analyzed. The data were normalized to the observation median, log-transformed, and standardized. If a compound was absent in a sample but present in the remaining replicates, it was considered a technical error, and imputation was performed using the KNN (k-nearest neighbors) method with the impute package [55]. Principal component analysis (PCA) was performed using pcaMethods [56]. For metabolite set enrichment analysis (MSEA), an over-representative analysis (ORA) was performed using a hypergeometric test. The Benjamini and Hochberg method was used to correct *p*-values for multiple testing [57]. Random Forest (RF) classification was performed using the RandomForest package. Mean decrease accuracy (MDA) was used as a variable importance measure [58]. Support vector machine (SVM) classification was performed in the rminer package [59]. Sensitivity analysis was used to determine variable importance [60].

3. Results

3.1. Field Phenotyping

Analysis of the traits concerning the yield revealed the differentiation of the sample by attitude to water supply. The most convincing character differing in genotypes in both regimes was seed productivity. Based on the quantitative value of this trait for two years, the genotypes were classified into three groups: drought-tolerant, drought-sensitive, and those forming approximately the same productivity under both regimes were considered indifferent (neutral) with respect to water supply. One, the most productive genotype in each regime for both years of experiment, was selected from each group (Table 2).

Table 2. Seed productivity (g/plant) of guar plants during two years on different regimes of water supply.

		2023		2024	
Genotype (VIR Number)	**Attitude to Water Supply**	**Drought**	**Watering (5.0 L/plant per week)**	**Drought**	**Watering (2.5 L/plant per week)**
k-52938	Drought-tolerant	35.1 ± 9.16	16.3 ± 6.41	21.1 ± 0.95	16.04 ± 2.47
k-52924	Drought-sensitive	12.2 ± 4.04	27.2 ± 4.17	6.1 ± 0.79	23.42 ± 2.23
k-52891	Neutral to water supply	19.8 ± 3.01	17.6 ± 4.62	10.34 ± 1.99	13.93 ± 5.05

3.2. Different Genotypes Exhibited Varying Responses to Drought Stress

GC-MS analysis yielded metabolite profiles (Figure 1) for tolerant, sensitive, and neutral genotypes grown under well-watered and drought conditions. The profiles included approximately 300 compounds; around 70 were identified by matching mass spectra and retention indices with library records, while the chemical class for about 150 mass spectra was annotated. Sugars and their derivatives were the most abundant in the obtained metabolite profiles. Additionally, the profiles contained approximately two dozen carboxylic acids, including intermediates of energy metabolism. Among the compounds

related to lipid metabolism, free fatty acids (FFAs), acylglycerols (AGs), and sterols were detected. Conversely, the profiles were poor in nitrogen-containing compounds.

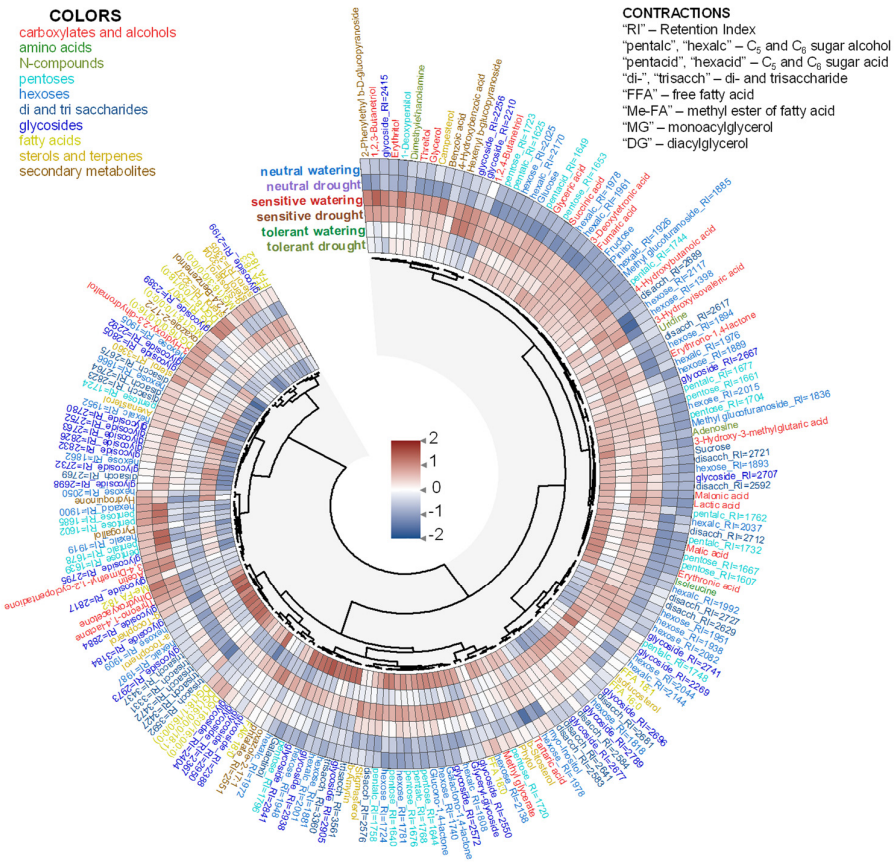


Figure 1. Heatmap of the mean metabolite content in the three genotypes of *Cyamopsis tetragonoloba* under watering and drought. Data normalized per sample median, log-transformed, and standardized. Metabolites are clustered by Pearson correlation and Ward method.

Quantitative analysis revealed a complex pattern of metabolite accumulation dependent on the drought resistance of the genotypes and the watering regime (Figure 1). Initially, dimension reduction was performed using unsupervised methods such as principal component analysis (PCA) and multidimensional scaling (MDS), using Spearman's distance (1-rho, where rho is Spearman's correlation coefficient) as a distance metric (Figure 2). In the resulting PCA model, the difference between the neutral genotype and the others was associated with the principal component (PC1), explaining 36.6% of the variance. Metabolite profiles of the drought-tolerant genotype also showed a clear difference between drought and watering conditions. This difference was related to PC2, explaining 16.4% of the variance. Examination of each genotype individually revealed that the clearest differences in metabolite profiles between plants under drought and well-watered conditions were observed in the tolerant variant (Figure 3a,b). In this case, the differences in plant profiles under normal and drought conditions were clearly distinguished by PC1, explaining 44% of the variance. In the case of the sensitive genotype (Figure 3c,d), the differences were not clear; the groups overlapped, but not significantly. The differences were mainly associated with PC1, which in this case explained 30% of the variance. The neutral variant (Figure 3e,f) showed the least pronounced differences between profiles of seeds from plants grown under drought and well-watered conditions. The groups showed an overlap, and the divergence was associated with PC2, explaining just 17% of the variance.

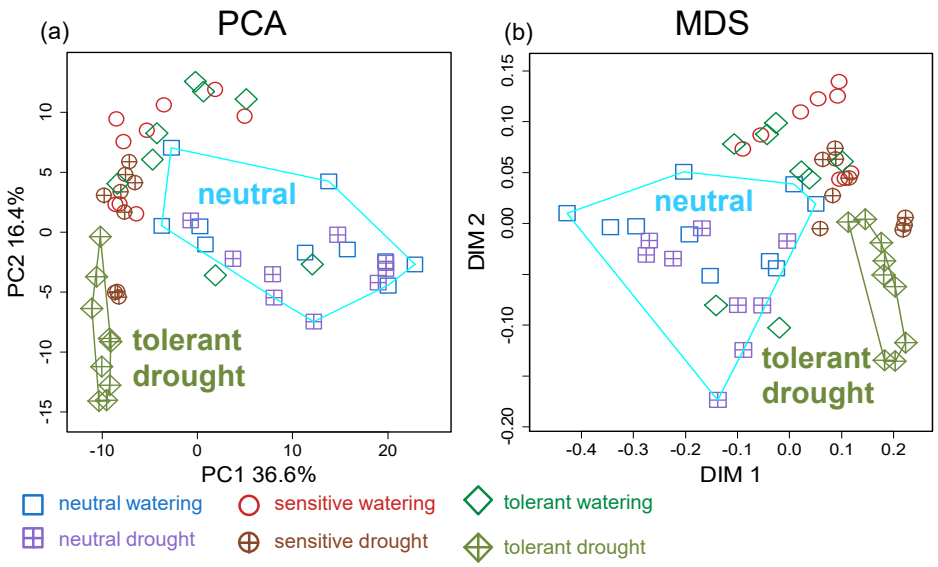


Figure 2. Ordination of metabolite profiles of guar seeds of tolerant, sensitive, and neutral genotypes grown under watering and drought. (**a**) PCA score plot, % is the percentage of variance related to the principal component (PC). (**b**) Multidimensional scaling (MDS) using 1-rho as a distance measure, where rho is the Spearman's correlation coefficient.

3.3. Impact of Drought on Metabolite Accumulation

To identify differentially accumulated metabolites (DAMs), random forest (RF) and support vector machine (SVM) methods were employed. The importance of metabolites in the constructed classification models was used to select metabolites as drought biomarkers. In the case of RF, MDA (mean decrease accuracy) was used. In the case of SVM, sensitivity analysis was employed to evaluate variable importance. Figure 4a shows a scatter plot of metabolites in the coordinates of variable importance from RF and SVM models for classifying profiles of tolerant genotype seeds grown under drought and well-watered conditions. As can be seen, a group of metabolites with high importance values in both models stands out. Moreover, for these metabolites, the Mann–Whitney–Wilcoxon test (MWW test) showed a very high significant difference ($p < 0.001$) in the levels of accumulation. Normalized accumulation levels of these metabolites are presented in Figure 4b. These are mainly sugars and their derivatives, with monosaccharides showing predominantly increased content under drought, and glycosides—a decrease. The second group of metabolites showed a slightly lower importance in RF, but the same high importance in SVM, as well as high significance in the MWW test ($0.001 < p < 0.01$). Figure 4c shows a heat map of their normalized content. This set also predominantly contains sugars and their derivatives, among which the sucrose content is increased under drought, while glucose and fructose did not show pronounced differences. Also, a greater accumulation of lactate and α-tocopherol under drought can be noted. To link the effect of drought with chemical classes of metabolites, we performed ORA (over-representation analysis, Figure 4d) for the set of metabolites shown in Figure 4b,c. The results confirmed that in the case of the tolerant genotype, the response to drought is related to the pattern of sugar accumulation. While the accumulation of glycosides and oligosaccharides grows and decreases equally, on the contrary, the accumulation of monosaccharides increases under drought conditions. The search for drought biomarkers in sensitive (Figure 5) and neutral (Figure 6) genotypes revealed only a small number of molecules whose accumulation is reliably associated with it. In both cases, these were trisaccharides.

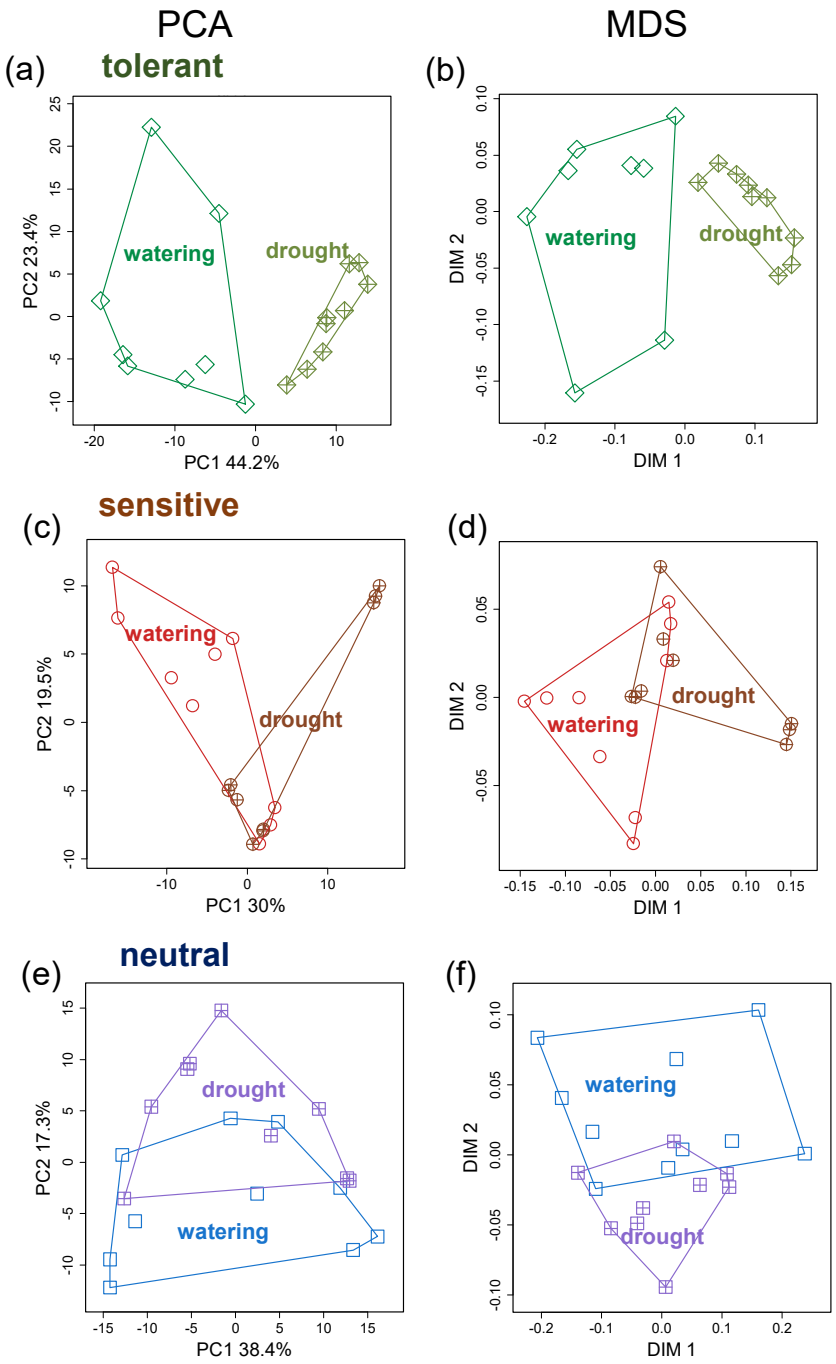


Figure 3. Metabolomic difference of guar seeds of tolerant (**a**,**b**), sensitive (**c**,**d**), and neutral (**e**,**f**) genotypes growing under watering and drought. (**a**,**c**,**e**) PCA score plot. (**b**,**d**,**f**) Scattering ordination by multidimensional scaling (MDS) using 1-rho as a distance measure, where rho is the Spearman's correlation coefficient.

To compare the effect of water regime on the metabolism of the three genotypes, we compared the logarithms of the fold changes in metabolite accumulation under drought and well-watered conditions $\log_2 \frac{drought}{well-watered}$. It turned out that the response to drought of tolerant and sensitive genotypes (Figure 5c) demonstrates a high correlation (rho = 0.78, $p < 10–16$). The tolerant genotype, however, shows stronger differences in the accumulation of metabolites between the two irrigation regimes. It is noteworthy that the greatest upregulation under drought was shown by several di- and trisaccharides, whereas the neutral genotype did not show a correlation in the response to drought with sensitive and tolerant ones.

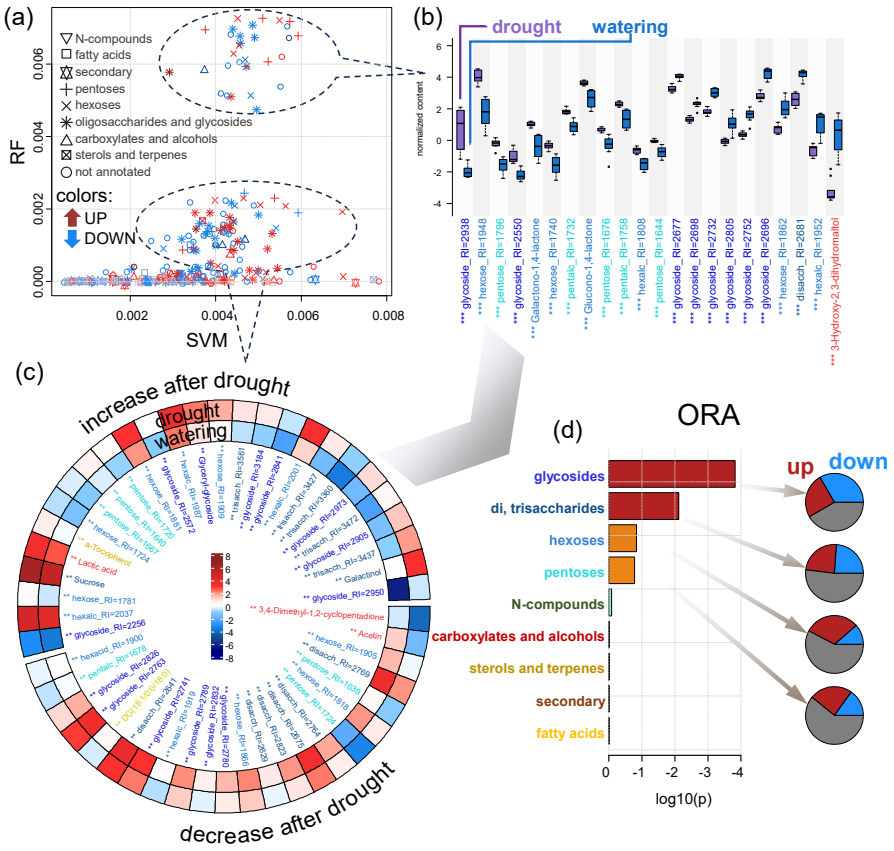


Figure 4. Selection of metabolomic features related to drought effects on seeds of tolerant genotype. (**a**) Scattering of metabolites in the space of the importances for classification models of support vector machine (SVM) and random forest (RF). (**b**) Boxplots of normalized content of the most important annotated metabolites. Red color corresponds to a higher level under drought and blue—under watering. (**c**) Heatmap of normalized content of less important metabolites. Asterisks represent adjusted p from MWW test: ***—$p < 0.001$, **—$p < 0.01$. (**d**) Overrepresentation analysis (ORA) of the selected features from (**b**,**c**). Pie charts represent proportions of selected metabolite which accumulation increased and decreased under drought.

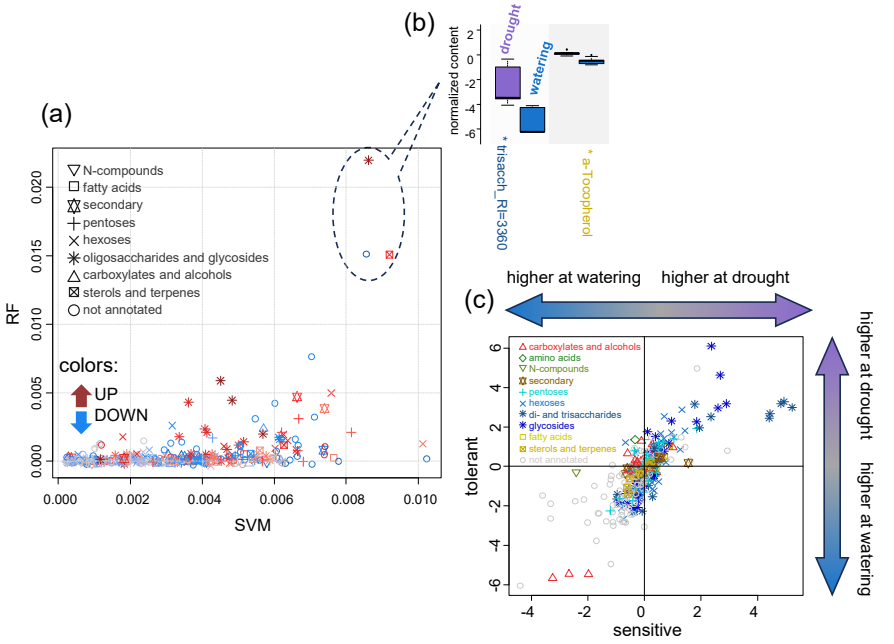


Figure 5. Selection of metabolomic features related to drought effects on seeds of a sensitive genotype. (**a**) Scattering of metabolites in the space of the importances for classification models of support vector

machine (SVM) and random forest (RF). (**b**) Boxplots of normalized content of most important metabolites. Asterisks * correspond to $0.01 < p < 0.05$. (**c**) Comparing of the water regime effects: scattering of metabolites in the space of logarithm of fold changes (FC) drought/watering for sensitive and stable variants.

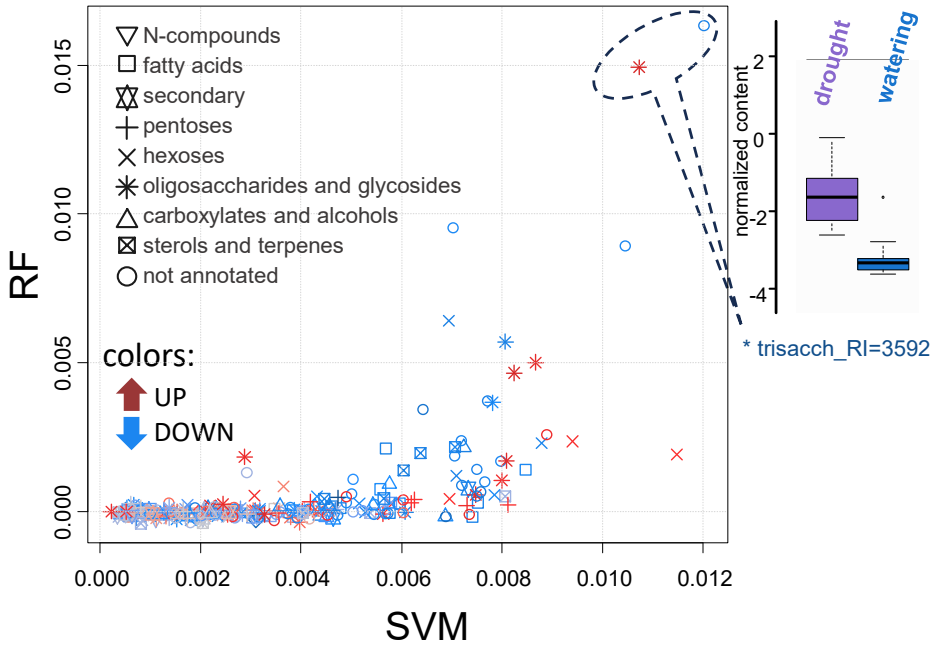


Figure 6. Selection of metabolomic features related to drought effects on seeds of neutral genotype. Scattering of metabolites in the space of the importances for classification models of support vector machine (SVM) and random forest (RF). Boxplots of normalized content of most important metabolites. Asterisks * correspond to $0.01 < p < 0.05$.

3.4. Genotype-Specific Differences Under Drought and Well-Watered Conditions

Ordination by PCA and MDS revealed that the three genotypes exhibit distinct metabolomic profiles. Under well-watering, the neutral and sensitive genotypes were most distant, while the tolerant genotype took an intermediate position. Drought enhanced the differences, particularly between the neutral genotype and the other two (Figure 7).

The results from the search for differentially accumulating metabolites in three genotypes by RF and MWW methods are presented on the heatmap, where the metabolites are clustered by the accumulation patterns similarity. Under well-watered conditions (Figure 8a), the neutral genotype exhibited elevated levels of lipophilic compounds (clusters I, III, IV) like acylglycerols and sterols, along with low accumulation of monosaccharides, TCA cycle intermediates (cluster VI), and secondary metabolites (cluster V). Conversely, tolerant and sensitive genotypes displayed higher levels of sugars and TCA cycle intermediates and related acids (cluster VI), including lactic, glyceric, fumaric, and malic acids, as well as numerous monosaccharides and sugar alcohols. The tolerant genotype also showed greater abundance of several sugars (cluster II), while the sensitive genotype was characterized by higher levels of aromatic compounds, certain glycosides, and sugar alcohols (cluster V).

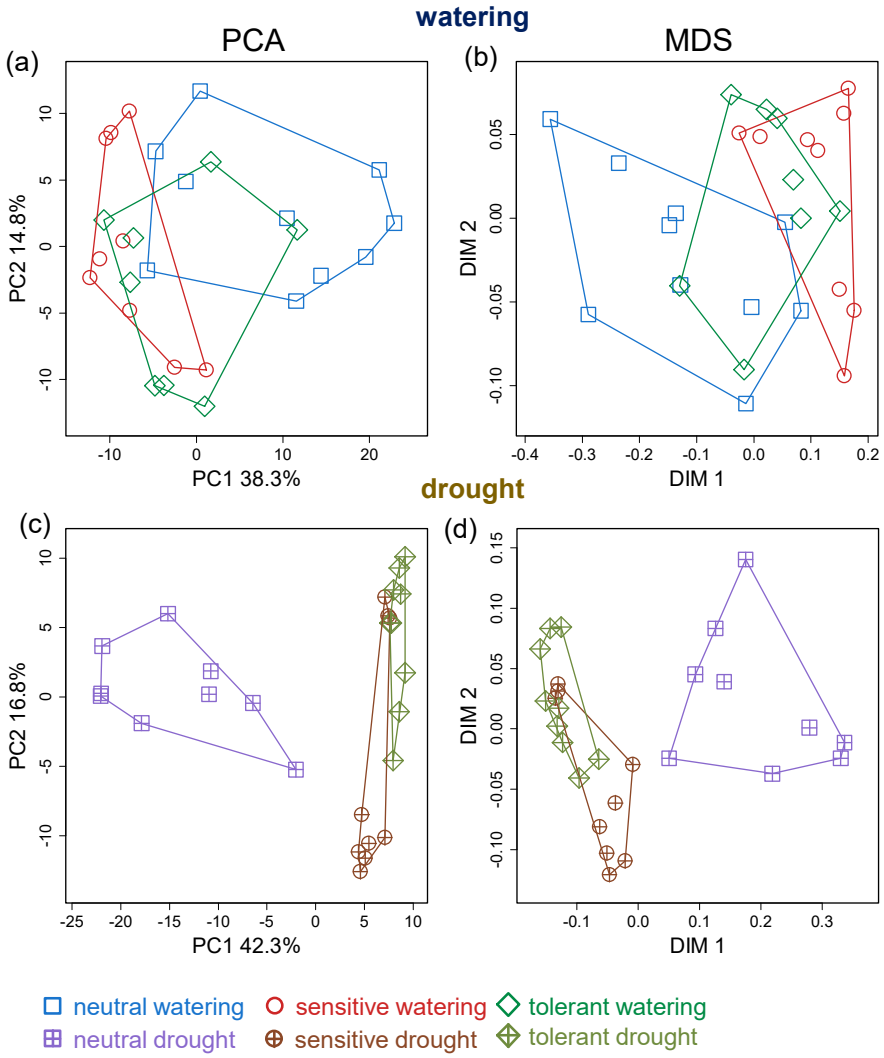


Figure 7. Metabolomic difference of seeds from the three guar genotypes under watering and drought. PCA score plots for watering (**a**) and drought (**b**) plants. Scattering in the coordinates extracted by MDS for watering (**c**) and drought (**d**).

Under drought, genotypic distinctions were more pronounced, with a greater number of metabolites showing differential accumulation (Figure 8b). The neutral genotype was characterized by higher accumulation of glycosides and pentoses (clusters I, II) coupled with reduced accumulation of numerous metabolites (clusters III, IV, VI). The tolerant genotype displayed a set of sugars and derivatives with increased accumulation (clusters V, VI), along with higher levels of tocopherols. The sensitive genotype exhibited greater accumulation of a specific set of sugars and derivatives, major phytosterols, and unsaturated C18 fatty acids. A large number of metabolites accumulated in higher levels in tolerant and sensitive genotypes compared to neutral (cluster IV), including sucrose, glucose, fructose, myo-inositol, TCA cycle intermediates, nitrogen-containing compounds, and β-sitosterol. Of the annotated metabolites, 41 showed genotype-specific differential accumulation under both drought and well-watered conditions (Figure 8c), with 86 differing only under drought and 12 showing accumulation only under well-watered conditions. Neutral and sensitive genotypes demonstrated similar differences under both conditions (Figure 9a, rho = 0.77). Differences between neutral and tolerant genotypes were less similar under two water regimes (Figure 9b, rho = 0.57). The differences between sensitive and tolerant genotypes under drought and well-watered conditions are only slightly similar (Figure 9c, rho = 0.22).

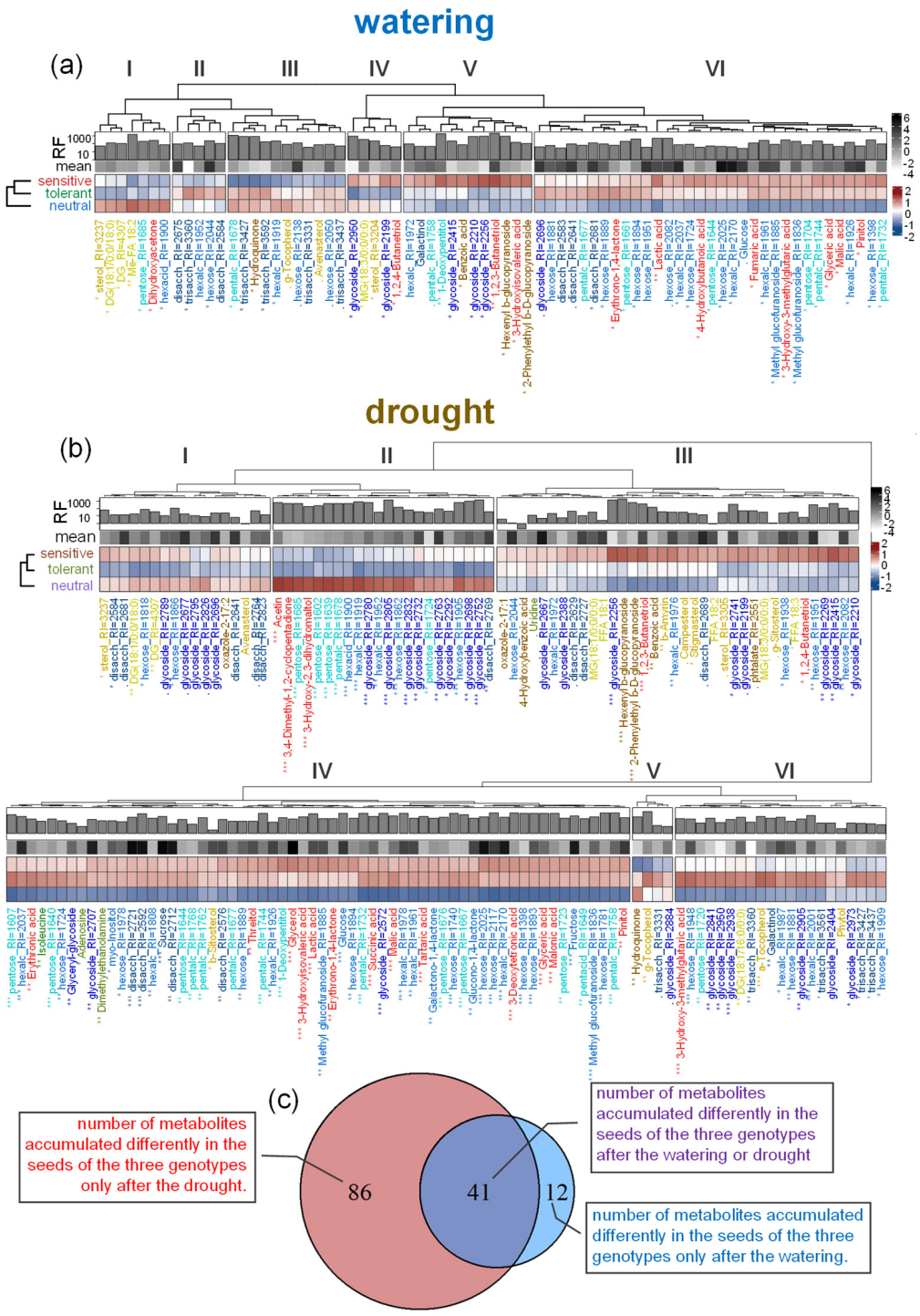


Figure 8. Metabolites accumulated differently in seeds of three guar genotypes under watering (**a**) and drought (**b**). Heatmap of normalized content of annotated metabolites selected by random forest. Asterisks represent adjusted *p* from MWW test: ***—$p < 0.001$, **—$p < 0.01$, *—$p < 0.05$, .—$p < 0.1$. (**c**) Venn diagram of metabolites differently accumulated (DAMs) in seeds of three genotypes under watering and drought. The Venn diagram shows the sets of differentially accumulated metabolites (DAMs) selected using the Kruskal–Wallis test (adjusted FDR < 0.05). The numbers represent the quantities of metabolites that accumulated specifically after drought (red area), watering (blue area), and both (intersection).

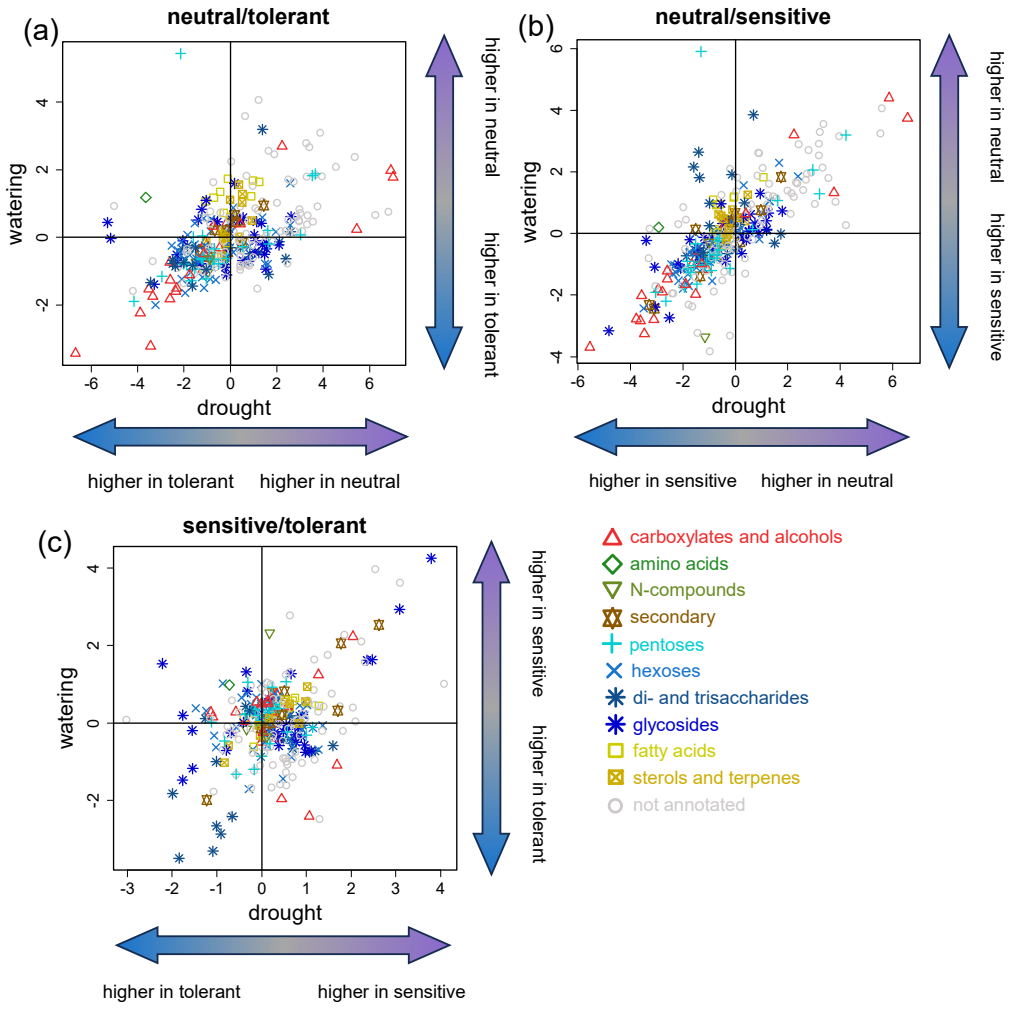


Figure 9. Comparison of the genotype-related metabolite differential accumulation under watering and drought. Scattering of metabolites in the space of logarithm of fold changes (FC): (**a**) neutral/tolerant, (**b**) neutral/sensitive, (**c**) sensitive/tolerant.

3.5. Metabolomic Divergence and Drought Response: Genotype-Specific Signatures

A comparative analysis of constitutive metabolite levels across the studied genotypes and their drought-induced metabolic shifts revealed limited or no correlation (Figure S1). However, the tolerant genotype exhibited a degree of correlation between its inherent metabolic signature and its response to drought. A moderate correlation (rho = 0.33, $p = 10^{-9}$) was observed between the differential metabolite accumulation between the tolerant and sensitive genotypes $\log_2 \frac{sensitive}{tolerant}$ and the drought response of the tolerant genotype $\log_2 \frac{tolerant-drought}{tolerant-well-watered}$. Scattering of metabolites in coordinates of log2(FC) illustrates this correlation (Figure 10). It should also be noted that three molecules (3-hydroxy-2,3-dihydromaltol, 3,4-dimethyl-1,2-cyclopentadione, and acetine) demonstrated a strong decrease in accumulation under drought and less accumulation less accumulation in seeds of the tolerant genotype.

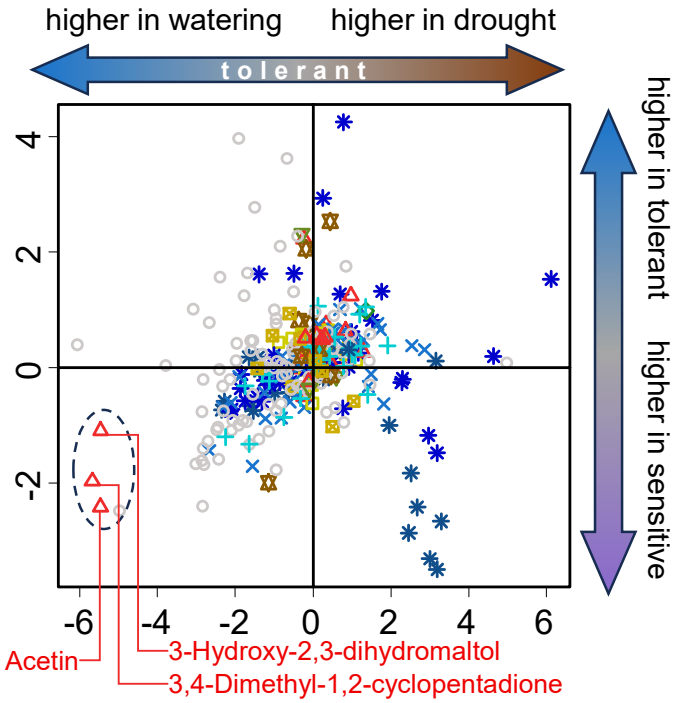


Figure 10. Comparison of the drought response of the tolerant genotype and differences of tolerant and sensitive genotypes. Scattering of metabolites in the space of logarithm of fold changes. Symbols are the same as at Figure 9.

3.6. Watering Regime Modulates Metabolite Correlation Patterns

To elucidate the influence of watering status and genetic background on the functional relationships between metabolite pools, we mapped metabolites, using significant (FDR < 0.05) correlation of its normalized content. The constructed graphs (Figure 11) consist of metabolites as nodes and correlations as edges, with positive correlations contracting nodes together. These networks differed markedly in both visual appearance and characteristics (Table S2). A common feature of all the graphs was a structure similar to a scale-free network, with a power-law-like distribution of node degrees. Also, networks demonstrated heterogeneity and a greater number of positive correlations. All three genotypes for drought cause an increased number of both positive and negative correlations. As a result, the average number of neighbors, network density, and clustering coefficient increased, while the average path length decreased. These effects were most prominent in the tolerant genotype. The ratio of positive to negative correlations decreased in the neutral genotype under drought, while it increased in the sensitive and tolerant genotypes. Network diameters and radii were relatively constant. A distinctive feature of the neutral genotype was its higher number of correlations.

The network structures varied considerably. In the neutral genotype, three distinct regions formed. Sugars, including almost all glycosides, are concentrated in the left large area. The second region contains free fatty acids, sterols, and other lipophilic compounds. Malate is also present in this region. During drought, malate is also present, and the hexose region is adjacent to this group. In the event of drought, this region is more isolated. The third region contains a large number of unannotated metabolites, as well as several sterols, including γ-sitosterol. In contrast, the networks of the sensitive and tolerant genotypes were notably heterogeneous, particularly under well-watered conditions. Moreover, the network structure differed more substantially in response to watering regime in these two genotypes. Cluster analysis, based on sets of edges, revealed that the neutral genotype differed from the other two. A separation based on the watering regime was observed in

the sensitive and neutral genotypes, suggesting a stronger impact of watering conditions on metabolic relations in these genotypes.

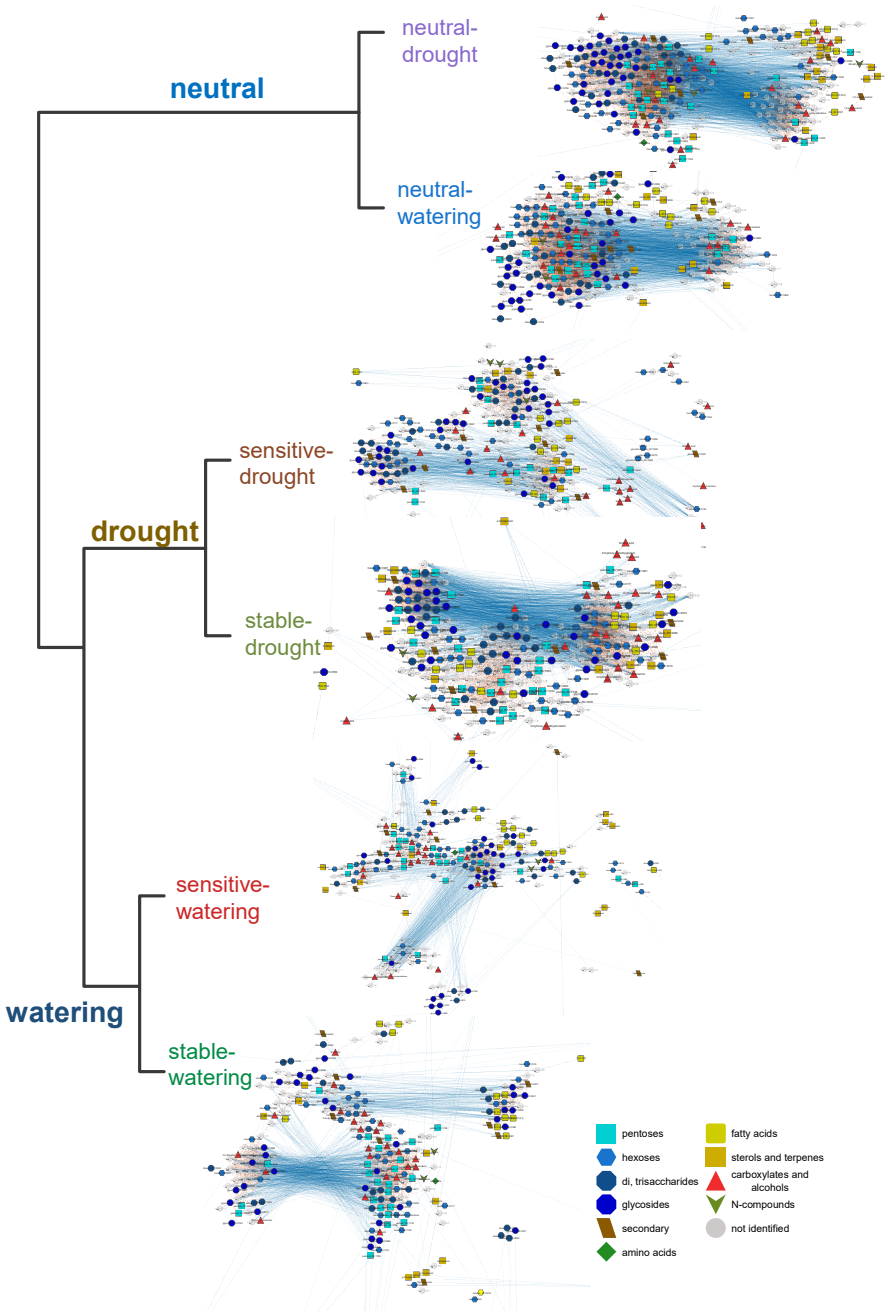


Figure 11. Mapping of metabolites by correlations of content (Spearman's correlation, FDR < 0.05). Nodes correspond to metabolites, shape and color reflect the class of the compound. The edges correspond to correlations, blue—negative, red—positive. Positive correlations constrict the nodes. Dendrogram of hierarchical clustering by the similarity of edges sets.

4. Discussion

4.1. General Trends in Response to Drought

In the experiment, the drought stress was applied to all genotypes for a fixed time (4 weeks). Generally, drought is known to affect plant developmental processes, slowing the growth and accelerating the leaf senescence [61,62]. Although we did not notice any differences between the studied genotypes regarding pod and seed appearance or size (both under drought and under normal watering), one should take into account the possibility

that the observed metabolic shifts might reflect differences in seed developmental stage (e.g., acceleration of seed maturation) rather than specific metabolic response to drought.

As shown by ordination (Figure 2), the tolerant genotype shows the most intense response to drought, indicating that the resistance of this genotype is determined by an active metabolic response. A more active stress response can be associated with greater resistance in other cases as well. For example, an intense response to drought was observed in the case of rapeseed seedlings of a resistant variety [63], and a more resistant melon variety showed greater changes in the metabolome in response to chilling [64]. A comparison of genotype responses to drought revealed similarities between tolerant and sensitive genotypes (Figure 5c). However, the response of tolerant genotypes was more intense. At the same time, the neutral genotype differed markedly from the others, although it demonstrated the weakest response to drought (Figure 3). Thus, the resistance of this genotype is determined by its constitutive specificity. However, its specific features did not show similarity with the response of the tolerant genotype (Figures 2 and S1). So, the constitutive features of the neutral genotype provide resistance by mechanisms other than the active response of the tolerant genotype.

Analysis of inter-varietal differences revealed that it was enhanced (Figure 7) and altered (Figure 9) by drought. To assess the influence of drought on intragenotype differences, a Venn diagram was constructed (Figure 8c, Table S3). Two sets of metabolites that accumulated differently in the three genotypes under watering and drought conditions were extracted using a Kruskal–Wallis test (FDR < 0.05). The number of metabolites whose accumulation differed between genotypes only after drought was 86. Under watering, this number was 12. The 41 metabolites were found to accumulate in varying amounts after being exposed to water or dry conditions. Thus, under drought, a greater number of metabolites showed differential accumulation in seeds belonging to different genotypes. This is probably the result of drought response features. Mainly, under drought, the difference between the neutral genotype and the other two is enhanced. This is the result of the response of tolerant and sensitive genotypes to drought in the absence of a pronounced response in the neutral genotype. It can also be noted that the neutral and sensitive genotypes differ the most under well-watering, while the tolerant genotype becomes the most distant from the neutral genotype under drought, which reflects its more intense response to drought.

4.2. Alteration of Sugar Accumulation Patterns: A Key Element of Drought Influence

Sugars and their derivatives were the dominant group of metabolites in the profiles. This pattern is common for plant metabolite profiles, including guar [65] and seeds in particular [66]. Sugars play a crucial role in the formation and maintenance of seed viability, providing cell resistance to desiccation [67]. Sugar accumulation is a key crosstalk of plant ontogenetic and stress responses. In all three genotypes, we observed a multitude of diverse carbohydrates that our analysis could not determine as specific molecules. This is not surprising, given that guar seeds accumulate galactomannan. Therefore, a high level of glycometabolism gene expression is observed in the endosperm during seed maturation [68]. A study of guar plant metabolite profiles in response to drought [38] showed that sugars were recognized as major classes of metabolites when analyzed by GC-MS metabolome analysis, where untargeted drought-responsive metabolites and biomarkers were proposed. Drought significantly influences carbohydrate accumulation, particularly in the tolerant genotype seeds. For example, sucrose content increases under drought in the tolerant genotype. Sugars, especially sucrose, are primary compounds responsible for osmotic pressure in plant cells [69]. Therefore, its accumulation may play a significant role in plant drought resistance. For example, it has been shown that drought increased the sucrose content in the

roots and leaves of rice. By contrast, the seeds of the tolerant genotype accumulated more sucrose, and this accumulation was activated more strongly after drought [70]. Another marker molecule whose increase was observed during drought in the tolerant genotype is galactinol. This compound is associated with raffinose metabolism. Plants can produce a variety of compounds that stabilize membranes and proteins. These include sugars. RFOs (raffinose family oligosaccharides) play a crucial role in stress responses. Their content increases in response to stress, including drought [71–73]. Galactinol and raffinose are involved in protecting plant cells from ROS [74,75]. Galactinol is important for maintaining seed vigor and longevity [75,76]. Galactinol synthase genes have been identified as markers of stress resistance in various legumes, such as peanuts [77], common bean [78], chickpea [75], and even in non-legumes, such as cucumber [79]. The precursor of galactinol is myo-inositol, the metabolism of which has been shown to increase during dehydration stress and plays an important role in seed physiology, seedling growth, and stress tolerance in chickpea [80]. In the seeds of sensitive and tolerant genotypes, its content is high, in contrast to the neutral genotype under drought. It can be assumed that the synthesis of galactinol from myo-inositol, characteristic of the tolerant genotype, causes, at least in part, the manifestation of the tolerance trait.

4.3. Differential Metabolite Accumulation as a Reflection of Destructive and Adaptive Processes

The differential accumulation of three compounds—3-hydroxy-2,3-dihydromaltol, 3,4-dimethyl-1,2-cyclopentadione, and acetine—is of particular interest. These molecules accumulate in the seeds of the drought-tolerant genotype in the smallest amount (Figures 4b,c, 8b and 10). The sensitive genotype also shows a decrease in their accumulation under drought, but not as sharp as in the tolerant one (Figure 5). Herewith, in the seeds of the neutral genotype, these molecules accumulated in large quantities both under irrigation and drought. These molecules are unusual for plant metabolite profiles. Isolated findings of acetine in plant profiles are known [81]. This molecule is an ester of glycerol and acetic acid. Acetine can be formed by acylation of glycerol with acid catalysis [82]. The reason for acidification and the source of acetate in plant tissues may be fermentation [23]. In this regard, it should be noted that the level of lactate in the seeds of sensitive and tolerant genotypes increased under drought. In addition, vacuoles are acidic compartments [83,84]. In turn, maltol and similar molecules are sometimes found in plant tissues [85,86]. Such molecules can be formed non-specifically, for example, during heat treatment of sugars in the presence of amino acids as a result of Maillard reactions [87]. 3,4-Dimethyl-1,2-cyclopentanedione, also known as caramel pentadione, belongs to the class of organic compounds known as cyclic ketones. This molecule is known to be formed during coffee roasting during Maillard reactions [88]. Similar processes of non-enzymatic glycation of proteins are reliably associated with stress [89–91]. Thus, it can be assumed that an important stress factor during drought is the induction of non-enzymatic chemical reactions. The response of tolerant and sensitive genotypes is the activation of mechanisms for their quenching, which is expressed as a decrease in the accumulation of their products. But, in the tolerant genotype, this reaction is more pronounced, which determines its higher resistance. Searching for markers of resistance and stress among low-molecular-weight products of non-enzymatic reactions may become a promising area of stress metabolomics. In addition, the observed low level of amino acids in seeds may be an adaptation that reduces the intensity of Maillard reactions. One of the mechanisms for quenching non-enzymatic reactions is the neutralization of ROS [92]. The accumulation of antioxidants such as tocopherols under drought in tolerant and sensitive genotypes is likely a manifestation of the operation of such a mechanism [93–96].

Accordingly, the increase in phytol content along with tocopherol may indicate the normal maturation of seeds in the tolerant genotype under drought conditions. It is known that phytol can be released during the degradation of chlorophyll [97], which occurs, among other things, during seed maturation [98]. There is a mention in the literature that in Brachypodium distachyon in leaves under drought, the content of phytol and tocopherol increases on days 8 and 16 of the drought treatment, which is associated with leaf senescence [99].

At the same time, the neutral genotype does not exhibit such reactions and does not suffer from high accumulation of non-enzymatic reaction products. The seeds of this genotype accumulate relatively large amounts of g-tocopherol hydroquinone. Hydroquinone is known for its antioxidant properties [100]. The accumulation of these molecules can be associated with their constitutive resistance.

The main source of ROS for heterotrophic tissues is respiration [101–104]. It follows that one way to minimize the generation of ROS under stress is to reduce the level of respiration. Interestingly, the neutral genotype, which showed the weakest reaction to drought, has a reduced level of TCA intermediates in seeds, regardless of the water regime. Perhaps a constitutively reduced level of seed respiration is an important factor determining the resistance of this genotype.

One of the key findings is the accumulation of free fatty acids (FFAs), acylglycerols (AGs), and sterols under stress. We propose that this process is an indicator of membrane degradation. It occurs as a result of lipid peroxidation (LPO), which is a consequence of the formation of ROS [105]. In addition, autophagy processes can be triggered to mobilize reserves, restructure cellular structures, and utilize damaged components [106–108].

It was the seeds of the sensitive genotype that were distinguished by a high accumulation of these molecules under drought (Figure 8b). This probably indicates a higher level of membrane degradation in the sensitive genotype. Also, some DAGs and sterols showed a trend towards relatively high accumulation in the seeds of the neutral genotype. Desiccation has a complex destructive effect on cell membranes, including the peroxidation and dehydration of polar phospholipid groups [109]. This, in turn, leads to membrane remodeling [110,111]. In plant cells, diacylglycerol (DG) plays a key intermediate role in the synthesis, catabolism, and conversion of glycerolipids. This establishes their role in membrane biogenesis and remodeling [112–118]. In addition, DGs could probably act as signal messengers modulating stress response [115,117,119]. As expected, DG content is known to respond to various developmental and environmental triggers [117,119]. Increased accumulation of DG was observed in response to several stress factors [120]. Based on this, it can be deduced that elevated DG levels in the neutral genotype (Figures 1 and 8) may allow for increased constitutive activity of lipid interconversion, ensuring flexible lipid metabolism under stressful conditions.

Further, abiotic stress is often accompanied by the accumulation of phytosterols. Higher sterol levels have also been linked to higher drought resistance [121,122]. Phytosterols are important for maintaining membrane fluidity, permeability, organization, and the regulation of transmembrane protein activity [123,124]. Conversely, decreased productivity in sensitive genotypes during drought, together with increased sterol and FFA levels, suggests that they are markers of stress processes in this case. Previously, an increase in sterol and FFA levels was observed in plant cell cultures in the late stages of development, when destructive processes began to occur [125]. Therefore, in this instance, the elevated phytosterol and FFA levels in the seeds of the sensitive genotype reflect the interconnected processes of membrane degradation and remodeling, which are insufficient to counteract the effects of the stressor.

Since drought, like other stress factors, can affect the quality of seed, it is interesting from the practical point of view whether the drought changes the accumulation of compounds with potential health effects when used as a food. In light of this, the accumulation of tocopherols and phytol in seeds of a tolerant guar genotype under drought may be beneficial for the food industry. Indeed, tocopherols belong to the Vitamin E group of nutrient compounds essential for humans [126]. Phytol and its metabolites are potential dietary compounds for cancer prevention [127]. However, given that guar seeds are primarily used to obtain gum (which is used as a food additive in ice cream or sauces [128]), and we were unable to identify the gum components in our analysis, the question remains open as to whether drought-resistant guar varieties may outperform the drought-sensitive ones in delivering health-promoting active compounds.

4.4. Influence of Drought on Functional Relationships of Metabolites

An important aspect of the systems approach is the analysis of rearrangements of interrelations between components. In our case, correlation can be used to assess how closely metabolites are related [129]. It has been shown that correlation patterns are specific to the genotype, including point mutations [130], and to environmental conditions [131,132]. Clustering of variants by similarity of correlations revealed that the neutral genotype turned out to be unlike the other two (Figure 11). These data correspond to the ordination of metabolite profiles (Figure 2). We mapped metabolites by significant correlations of their content (Figure 11). The resulting graphs differed both in appearance and in characteristics (Table S2). This indicates significant functional differences between the variants. Importantly, drought led to an increase in the number of correlations, both positive and negative, resulting in smaller diameters and radii of networks and an increase in their density. Correlations are the result of a combination of all reactions and regulatory processes [133–135]. This suggests tighter co-regulation of metabolite accumulation, likely determined by the development of a stress response. Such an effect of stress has been observed previously [49]. Nevertheless, the effect of drought on the correlation pattern among the three genotypes was the smallest in the neutral genotype. Also, in the case of the neutral genotype, the proportion of negative correlations increased. Negative correlations between metabolite levels are determined by the law of conservation of energy [133]. As the system has less resources, this should lead to an increase in negative correlations. It can be assumed that the increase in the proportion of negative correlations is associated with a deterioration of conditions in an unfavorable environment in the absence of a pronounced reaction. This is consistent with the fact that, as mentioned above, the neutral genotype is prone to the accumulation of metabolites associated with stress. In the case of tolerant and sensitive genotypes, the decisive factor determining the pattern of metabolite connections was drought. Interestingly, unlike the neutral, in the case of sensitive and tolerant genotypes, an increase in the proportion of positive correlations was observed. The reason for the high positive correlation may be proximity to equilibrium and a high level of metabolic flow [133]. This may be the result of intense metabolism associated with a more active response to stress.

5. Conclusions

Metabolite profiling of seeds from three varieties with different drought responses showed that the neutral genotype differs sharply from the sensitive and tolerant types in terms of both metabolite accumulation and the correlation pattern of their content. The neutral genotype, showing stable productivity, also showed a lower effect of drought on the seed metabolome. Probably, its resistance is associated with constitutive features of metabolism, e.g., elevated DG levels, which likely ensures increased lipid interconversion

under stressful conditions, resulting in reduced respiration intensity. In turn, the tolerant genotype, showing an increase in productivity under drought, also demonstrated the most striking response. Therefore, the tolerant genotype J.C.3118 is preferable for breeding work aimed at creating drought-tolerant guar varieties. The responses of the sensitive and tolerant genotypes to drought are qualitatively similar, but the reaction of the first is less intense. Conversely, the negative impact of drought on the sensitive genotype may be determined by the insufficiency of the antioxidant system, as indicated by relatively low levels of hydroquinone and tocopherols. The response to drought primarily consists of changing the pattern of accumulation of sugars and their derivatives. An important consequence of drought is intensifying differences between genotypes. Such effects can be used in the future for better phenotypic differentiation of genotypes.

Drought leads to a significant rearrangement of the functional connections of metabolites in tolerant and sensitive genotypes. This is probably due to the important role of carbohydrates, such as galactomannans, in the metabolism of guar seeds. A likely mechanism of resistance of the tolerant genotype is the activation of quenching of non-enzymatic reactions. The tolerant genotype demonstrated a significant decrease in the potential products of these reactions in the seeds under drought conditions. Among the candidates for stress and resistance markers, the most promising are low-molecular-weight products of non-enzymatic reactions, antioxidants, free fatty acids, sterols, and metabolites of chlorophyll metabolism such as phytol. Thus, the content of galactinol and, possibly, the level of expression of galactinol biosynthesis genes may be suitable biomarkers of the trait of guar tolerance to drought. Transcriptomic and proteomic studies can expand and refine the mechanisms of drought influence on guar seeds. Integration of metabolomics, transcriptomics, and proteomics can allow us to model in detail the mechanisms of guar resistance to drought. Further work will be aimed at analyzing transcriptomic changes in maturing seeds of these genotypes in order to move from metabolomic markers to transcriptomic and DNA markers. This will subsequently ensure the improvement of techniques for breeding new resistant lines.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15122778/s1. Supplementary S1: metabolomic dataset: Table S1. extracted peak areas. Supplementary S2: Table S2. Correlation networks parameters. Supplementary S3: Figure S1. Comparing drought response and differences of genotypes. Supplementary S4: Table S3. Marker metabolites of seeds from tolerant, neutral and sensitive genotypes.

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References

1. Thombare, N.; Jha, U.; Mishra, S.; Siddiqui, M.Z. Guar Gum as a Promising Starting Material for Diverse Applications: A Review. *Int. J. Biol. Macromol.* **2016**, *88*, 361–372. [CrossRef]
2. *GUAR MARKET SIZE & SHARE ANALYSIS—GROWTH TRENDS & FORECASTS (2025–2030)*; Mordor Intelligence Research&Advisory: Hyderabad, India. 2025. Available online: https://www.mordorintelligence.com/industry-reports/guar-market (accessed on 5 November 2025).
3. Dzyubenko, N.I.; Dzyubenko, E.A.; Potokina, E.K.; Bulyntsev, S.V. Guar *Cyamopsis tetragonoloba* (L.) Taub.: Characteristics, Use, Genetic Resources, and Potential for Introduction in Russia. *Agric. Biol.* **2017**, *52*, 1116–1128.
4. Kuravadi, N.A.; Verma, S.; Pareek, S.; Gahlot, P.; Kumari, S.; Tanwar, U.K.; Bhatele, P.; Choudhary, M.; Gill, K.S.; Pruthi, V.; et al. Guar. In *Agricultural Sustainability*; Elsevier: Amsterdam, The Netherlands, 2013; pp. 47–60, ISBN 978-0-12-404560-6.
5. Sindhuja, S. Trends and Economic Dynamics of Guar in India. *Econ. Aff.* **2022**, *67*, 769–778. [CrossRef]
6. Anderson, E. *Introgressive Hybridization*; Biological research series; J. Wiley: Hoboken, NJ, USA, 1949.
7. Vishnyakova, M.A.; Dzyubenko, E.A.; Kocherina, N.V.; Agakhanov, M.M.; Shaukharov, R.A. Phenotyping of Guar Accessions from the VIR Collection under Irrigation and Artificial Drought Conditions in Volgograd Province. *Proc. Appl. Bot. Genet. Breed.* **2025**, *186*, 93–105. [CrossRef]
8. Stafford, R.E.; McMichael, B.L.; Burke, J.J. Genetic Variation for Stress-Related Traits in Guar. *Environ. Exp. Bot.* **1991**, *31*, 303–312. [CrossRef]
9. Alshameri, A.; Al-Qurainy, F.; Khan, S.; Nadeem, M.; Gaafar, A.-R.; Alameri, A.; Tarroum, M.; Alansi, S.; Ashraf, M. Morpho-Physiological Responses of Guar [*Cyamopsis tetragonoloba* (L.) Taub.] to Multiple Stresses of Drought, Heat and Salinity. *Pak. J. Bot.* **2019**, *51*, 817–822. [CrossRef] [PubMed]
10. Gresta, F.; Mercati, F.; Santonoceto, C.; Abenavoli, M.R.; Ceravolo, G.; Araniti, F.; Anastasi, U.; Sunseri, F. Morpho-Agronomic and AFLP Characterization to Explore Guar (*Cyamopsis tetragonoloba*, L.) Genotypes for the Mediterranean Environment. *Ind. Crops Prod.* **2016**, *86*, 23–30. [CrossRef]
11. Avola, G.; Riggi, E.; Trostle, C.; Sortino, O.; Gresta, F. Deficit Irrigation on Guar Genotypes (*Cyamopsis tetragonoloba* (L.) Taub.): Effects on Seed Yield and Water Use Efficiency. *Agronomy* **2020**, *10*, 789. [CrossRef]
12. Venkateswarlu, B.; Rao, A.V.; Lahiri, A.N. Effect of Water Stress on Nodulation and Nitrogenase Activity of Guar (*Cyamopsis tetragonoloba* (L.) Taub.). *Proc. Indian Acad. Sci.* **1983**, *92*, 297–301. [CrossRef]
13. Venkateswarlu, B.; Rao, A.V. Quantitative Effects of Field Water Deficits on $N_2(C_2H_2)$ Fixation in Selected Legumes Grown in the Indian Desert. *Biol. Fertil. Soils* **1987**, *5*, 18–22. [CrossRef]
14. Shrestha, R.; Adams, C.B.; Ravelombola, W.; MacMillan, J.; Trostle, C.; Ale, S.; Hinson, P. Exploring Phenotypic Variation and Associations in Root Nodulation, Morphological, and Growth Character Traits among 50 Guar Genotypes. *Ind. Crops Prod.* **2021**, *171*, 113831. [CrossRef]
15. Ashraf, M.Y.; Akhtar, K.; Sarwar, G.; Ashraf, M. Evaluation of Arid and Semi-Arid Ecotypes of Guar (*Cyamopsis tetragonoloba* L.) for Salinity (NaCl) Tolerance. *J. Arid Environ.* **2002**, *52*, 473–482. [CrossRef]
16. Meftahizadeh, H.; Ghorbanpour, M.; Asareh, M.H. Comparison of Morphological and Phytochemical Characteristics in Guar (*Cyamopsis tetragonoloba* L.) Landraces and Cultivars under Different Sowing Dates in an Arid Environment. *Ind. Crops Prod.* **2019**, *140*, 111606. [CrossRef]
17. Pandey, K.; Kumar, R.S.; Prasad, P.; Sushma; Pande, V.; Trivedi, P.K.; Shirke, P.A. Synchronised Interaction of Carbon and Nitrogen Provides Drought Tolerance in *Cyamopsis tetragonoloba*. *Environ. Exp. Bot.* **2022**, *199*, 104899. [CrossRef]
18. Reddy, B.M.; Anthony Johnson, A.M.; Jagadeesh Kumar, N.; Venkatesh, B.; Jayamma, N.; Pandurangaiah, M.; Sudhakar, C. De Novo Transcriptome Analysis of Drought-Adapted Cluster Bean (Cultivar RGC-1025) Reveals the Wax Regulatory Genes Involved in Drought Resistance. *Front. Plant Sci.* **2022**, *13*, 868142. [CrossRef] [PubMed]
19. Vishnyakova, M.A.; Frolova, N.; Frolov, A. Drought Stress Response in Guar (*Cyamopsis tetragonoloba* (L.) Taub): Physiological and Molecular Genetic Aspects. *Plants* **2023**, *12*, 3955. [CrossRef]
20. Grierson, C.S.; Barnes, S.R.; Chase, M.W.; Clarke, M.; Grierson, D.; Edwards, K.J.; Jellis, G.J.; Jones, J.D.; Knapp, S.; Oldroyd, G.; et al. One Hundred Important Questions Facing Plant Science Research. *New Phytol.* **2011**, *192*, 6–12. [CrossRef]
21. Obata, T.; Fernie, A.R. The Use of Metabolomics to Dissect Plant Responses to Abiotic Stresses. *Cell. Mol. Life Sci.* **2012**, *69*, 3225–3243. [CrossRef]
22. Puzanskiy, R.K.; Yemelyanov, V.V.; Shishova, M.F. Metabolomics as a Modern Approach for the Investigation of Potato Plant Adaptation to Biotic and Abiotic Stress Factors (Review). *Agric. Biol.* **2018**, *53*, 15–28.
23. Yemelyanov, V.V.; Puzanskiy, R.K.; Shishova, M.F. Plant Life with and without Oxygen: A Metabolomics Approach. *Int. J. Mol. Sci.* **2023**, *24*, 16222. [CrossRef]
24. Salam, U.; Ullah, S.; Tang, Z.-H.; Elateeq, A.A.; Khan, Y.; Khan, J.; Khan, A.; Ali, S. Plant Metabolomics: An Overview of the Role of Primary and Secondary Metabolites against Different Environmental Stress Factors. *Life* **2023**, *13*, 706. [CrossRef]

25. Głuchowska, A.; Zieniuk, B.; Pawełkowicz, M. Unlocking Plant Resilience: Metabolomic Insights into Abiotic Stress Tolerance in Crops. *Metabolites* **2025**, *15*, 384. [CrossRef]
26. Weckwerth, W.; Loureiro, M.E.; Wenzel, K.; Fiehn, O. Differential Metabolic Networks Unravel the Effects of Silent Plant Phenotypes. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 7809–7814. [CrossRef] [PubMed]
27. Dobson, G.; Shepherd, T.; Verrall, S.R.; Griffiths, W.D.; Ramsay, G.; McNicol, J.W.; Davies, H.V.; Stewart, D. A Metabolomics Study of Cultivated Potato (*Solanum tuberosum*) Groups Andigena, Phureja, Stenotomum, and Tuberosum Using Gas Chromatography−Mass Spectrometry. *J. Agric. Food Chem.* **2010**, *58*, 1214–1223. [CrossRef] [PubMed]
28. Hamany Djande, C.Y.; Pretorius, C.; Tugizimana, F.; Piater, L.A.; Dubery, I.A. Metabolomics: A Tool for Cultivar Phenotyping and Investigation of Grain Crops. *Agronomy* **2020**, *10*, 831. [CrossRef]
29. Fernie, A.R.; Schauer, N. Metabolomics-Assisted Breeding: A Viable Option for Crop Improvement? *Trends Genet.* **2009**, *25*, 39–48. [CrossRef]
30. Sharma, V.; Gupta, P.; Priscilla, K.; SharanKumar, S.; Hangargi, B.; Veershetty, A.; Ramrao, D.P.; Suresh, S.; Narasanna, R.; Naik, G.R.; et al. Metabolomics Intervention Towards Better Understanding of Plant Traits. *Cells* **2021**, *10*, 346. [CrossRef]
31. Hill, C.B.; Taylor, J.D.; Edwards, J.; Mather, D.; Langridge, P.; Bacic, A.; Roessner, U. Detection of QTL for Metabolic and Agronomic Traits in Wheat with Adjustments for Variation at Genetic Loci That Affect Plant Phenology. *Plant Sci.* **2015**, *233*, 143–154. [CrossRef]
32. Yin, B.; Jia, J.; Sun, X.; Hu, X.; Ao, M.; Liu, W.; Tian, Z.; Liu, H.; Li, D.; Tian, W.; et al. Dynamic Metabolite QTL Analyses Provide Novel Biochemical Insights into Kernel Development and Nutritional Quality Improvement in Common Wheat. *Plant Commun.* **2024**, *5*, 100792. [CrossRef]
33. Razzaq, A.; Sadia, B.; Raza, A.; Khalid Hameed, M.; Saleem, F. Metabolomics: A Way Forward for Crop Improvement. *Metabolites* **2019**, *9*, 303. [CrossRef]
34. Hussain, S.; Hussain, S.; Qadir, T.; Khaliq, A.; Ashraf, U.; Parveen, A.; Saqib, M.; Rafiq, M. Drought Stress in Plants: An Overview on Implications, Tolerance Mechanisms and Agronomic Mitigation Strategies. *Plant Sci. Today* **2019**, *6*, 389–402. [CrossRef]
35. Mata, A.T.; Jorge, T.F.; Pires, M.V.; Antonio, C. Drought Stress Tolerance in Plants: Insights from Metabolomics. In *Drought Stress Tolerance in Plants*; Hossain, M.A., Wani, S.H., Bhattacharjee, S., Burritt, D.J., Tran, L.-S.P., Eds.; Springer International Publishing: Cham, Switzerland, 2016; Volume 2, pp. 187–216. ISBN 978-3-319-32421-0.
36. Raza, A.; Anas, M.; Bhardwaj, S.; Mir, R.A.; Charagh, S.; Elahi, M.; Zhang, X.; Mir, R.R.; Weckwerth, W.; Fernie, A.R.; et al. Harnessing Metabolomics for Enhanced Crop Drought Tolerance. *Crop J.* **2025**, *13*, 311–327. [CrossRef]
37. Frank, T.; Engel, K.-H. Metabolomic Analysis of Plants and Crops. In *Metabolomics in Food and Nutrition*; Elsevier: Amsterdam, The Netherlands, 2013; pp. 148–191. ISBN 978-1-84569-512-5.
38. Sharma, S.; Kumar, M.; Sircar, D.; Prasad, R. Metabolic Profiling and Biomarkers Identification in Cluster Bean under Drought Stress Using GC-MS Technique. *Metabolomics* **2024**, *20*, 80. [CrossRef] [PubMed]
39. Lin, H.; Rao, J.; Shi, J.; Hu, C.; Cheng, F.; Wilson, Z.A.; Zhang, D.; Quan, S. Seed Metabolomic Study Reveals Significant Metabolite Variations and Correlations among Different Soybean Cultivars. *J. Integr. Plant Biol.* **2014**, *56*, 826–836. [CrossRef] [PubMed]
40. Smolikova, G.N.; Shavarda, A.L.; Alekseichuk, I.V.; Chantseva, V.V.; Medvedev, S.S. The Metabolomic Approach to the Assessment of Cultivar Specificity of Brassica Napus L. Seeds. *Vavilov J. Genet. Breed.* **2015**, *19*, 121. [CrossRef]
41. Ning, K.; Hou, C.; Wei, X.; Zhou, Y.; Zhang, S.; Chen, Y.; Yu, H.; Dong, L.; Chen, S. Metabolomics Analysis Revealed the Characteristic Metabolites of Hemp Seeds Varieties and Metabolites Responsible for Antioxidant Properties. *Front. Plant Sci.* **2022**, *13*, 904163. [CrossRef]
42. Othibeng, K.; Nephali, L.; Tugizimana, F. Seed Metabolomic Landscape Reflecting Key Differential Metabolic Profiles Among Different Wheat Cultivars. *Metabolites* **2025**, *15*, 603. [CrossRef]
43. Leonova, T.S.; Bilova, T.E.; Frolov, A.A. Models of Osmotic Stress as a Tool for Proteomics and Metabolomics of Legume Seeds. *Ecol. Genet.* **2024**, *22*, 205–220. [CrossRef]
44. Wolfender, J.-L.; Rudaz, S.; Hae Choi, Y.; Kyong Kim, H. Plant Metabolomics: From Holistic Data to Relevant Biomarkers. *Curr. Med. Chem.* **2013**, *20*, 1056–1090. [CrossRef]
45. Ma, A.; Qi, X. Mining Plant Metabolomes: Methods, Applications, and Perspectives. *Plant Commun.* **2021**, *2*, 100238. [CrossRef]
46. Galal, A.; Talal, M.; Moustafa, A. Applications of Machine Learning in Metabolomics: Disease Modeling and Classification. *Front. Genet.* **2022**, *13*, 1017340. [CrossRef] [PubMed]
47. Greenacre, M.; Groenen, P.J.F.; Hastie, T.; D'Enza, A.I.; Markos, A.; Tuzhilina, E. Principal Component Analysis. *Nat. Rev. Methods Primer* **2022**, *2*, 100. [CrossRef]
48. Yuan, X.; Smith, N.S.S.; Moghe, G.D. Analysis of Plant Metabolomics Data Using Identification-free Approaches. *Appl. Plant Sci.* **2025**, *13*, e70001. [CrossRef]
49. Yurkov, A.P.; Puzanskiy, R.K.; Kryukov, A.A.; Kudriashova, T.R.; Kovalchuk, A.I.; Gorenkova, A.I.; Bogdanova, E.M.; Laktionov, Y.V.; Romanyuk, D.A.; Yemelyanov, V.V.; et al. The Effect of Arbuscular Mycorrhizal Fungus and Phosphorus Treatment on Root Metabolome of Medicago Lupulina During Key Stages of Development. *Plants* **2025**, *14*, 2685. [CrossRef]

50. Heinemann, J.; Mazurie, A.; Tokmina-Lukaszewska, M.; Beilman, G.J.; Bothner, B. Application of Support Vector Machines to Metabolomics Experiments with Limited Replicates. *Metabolomics* **2014**, *10*, 1121–1128. [CrossRef]
51. Xue, W.; Li, F.; Li, X.; Liu, Y. A Support Vector Machine-Assisted Metabolomics Approach for Non-Targeted Screening of Multi-Class Pesticides and Veterinary Drugs in Maize. *Molecules* **2024**, *29*, 3026. [CrossRef]
52. Breiman, L. Random Forests. *Mach. Learn.* **2001**, *45*, 5–32. [CrossRef]
53. Quintanilla-Casas, B.; Bro, R.; Hinrich, J.L.; Davie-Martin, C.L. Tutorial on PARADISe: PARAFAC2-Based Deconvolution and Identification System for Processing GC–MS Data 2023. 2024. Available online: https://slack.protocols.io:8443/view/tutorial-on-paradise-parafac2-based-deconvolution-dfda3i2e.html (accessed on 5 November 2025).
54. Hummel, J.; Selbig, J.; Walther, D.; Kopka, J. The Golm Metabolome Database: A Database for GC-MS Based Metabolite Profiling. In *Metabolomics*; Nielsen, J., Jewett, M.C., Eds.; Topics in Current Genetics; Springer: Berlin, Heidelberg, 2007; Volume 18, pp. 75–95. ISBN 978-3-540-74718-5.
55. Hastie, T.; Tibshirani, R.; Narasimhan, B.; Chu, G. Impute: Imputation for microarray data. 2023. Available online: https://doi.org/10.18129/B9.bioc.impute (accessed on 5 November 2025).
56. Stacklies, W.; Redestig, H.; Scholz, M.; Walther, D.; Selbig, J. pcaMethods—A Bioconductor Package Providing PCA Methods for Incomplete Data. *Bioinformatics* **2007**, *23*, 1164–1167. [CrossRef]
57. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Stat. Methodol.* **1995**, *57*, 289–300. [CrossRef]
58. Bénard, C.; Da Veiga, S.; Scornet, E. Mean Decrease Accuracy for Random Forests: Inconsistency, and a Practical Solution via the Sobol-MDA. *Biometrika* **2022**, *109*, 881–900. [CrossRef]
59. Cristianini, N.; Shawe-Taylor, J. *An Introduction to Support Vector Machines: And Other Kernel-Based Learning Methods*; Cambridge University Press: Cambridge, UK, 2000; ISBN 978-0-521-78019-3.
60. Cortez, P.; Embrechts, M.J. Using Sensitivity Analysis and Visualization Techniques to Open Black Box Data Mining Models. *Inf. Sci.* **2013**, *225*, 1–17. [CrossRef]
61. Seleiman, M.F.; Al-Suhaibani, N.; Ali, N.; Akmal, M.; Alotaibi, M.; Refay, Y.; Dindaroglu, T.; Abdul-Wajid, H.H.; Battaglia, M.L. Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. *Plants* **2021**, *10*, 259. [CrossRef]
62. Tan, S.; Sha, Y.; Sun, L.; Li, Z. Abiotic Stress-Induced Leaf Senescence: Regulatory Mechanisms and Application. *Int. J. Mol. Sci.* **2023**, *24*, 11996. [CrossRef]
63. Zhi, X.; Bian, X.; Yu, J.; Xiao, X.; Duan, B.; Huang, F.; Jiang, Z.; Zhou, G.; Ma, N. Comparative Metabolomics Analysis of Tolerant and Sensitive Genotypes of Rapeseed (*Brassica napus* L.) Seedlings under Drought Stress. *Agric. Water Manag.* **2024**, *296*, 108797. [CrossRef]
64. Diao, Q.; Tian, S.; Cao, Y.; Yao, D.; Fan, H.; Jiang, X.; Zhang, W.; Zhang, Y. Physiological, Transcriptomic, and Metabolomic Analyses of the Chilling Stress Response in Two Melon (*Cucumis melo* L.) Genotypes. *BMC Plant Biol.* **2024**, *24*, 1074. [CrossRef]
65. Grigoreva, E.; Tkachenko, A.; Arkhimandritova, S.; Beatovic, A.; Ulianich, P.; Volkov, V.; Karzhaev, D.; Ben, C.; Gentzbittel, L.; Potokina, E. Identification of Key Metabolic Pathways and Biomarkers Underlying Flowering Time of Guar (*Cyamopsis tetragonoloba* (L.) Taub.) via Integrated Transcriptome-Metabolome Analysis. *Genes* **2021**, *12*, 952. [CrossRef] [PubMed]
66. Rajaprakasam, S.; Rahman, H.; Karunagaran, S.; Bapu, K.; J.R.; Kulandivelu, G.; Kambale, R.; Ramanathan, V.; Muthurajan, R. Comparative Transcriptome and Metabolome Profiling in the Maturing Seeds of Contrasting Cluster Bean (*Cyamopsis tetragonoloba* L. Taub) Cultivars Identified Key Molecular Variations Leading to Increased Gum Accumulation. *Gene* **2021**, *791*, 145727. [CrossRef] [PubMed]
67. Smolikova, G.; Leonova, T.; Vashurina, N.; Frolov, A.; Medvedev, S. Desiccation Tolerance as the Basis of Long-Term Seed Viability. *Int. J. Mol. Sci.* **2020**, *22*, 101. [CrossRef] [PubMed]
68. Hu, H.; Wang, H.; Zhang, Y.; Kan, B.; Ding, Y.; Huang, J. Characterization of Genes in Guar Gum Biosynthesis Based on Quantitative RNA-Sequencing in Guar Bean (*Cyamopsis tetragonoloba*). *Sci. Rep.* **2019**, *9*, 10991. [CrossRef]
69. Beauvoit, B.P.; Colombié, S.; Monier, A.; Andrieu, M.-H.; Biais, B.; Bénard, C.; Chéniclet, C.; Dieuaide-Noubhani, M.; Nazaret, C.; Mazat, J.-P.; et al. Model-Assisted Analysis of Sugar Metabolism throughout Tomato Fruit Development Reveals Enzyme and Carrier Properties in Relation to Vacuole Expansion. *Plant Cell* **2014**, *26*, 3224–3242. [CrossRef]
70. Thomas, A.; Beena, R.; Laksmi, G.; Soni, K.B.; Alex, S.; Viji, M.M. Changes in Sucrose Metabolic Enzymes to Water Stress in Contrasting Rice Genotypes. *Plant Stress* **2022**, *5*, 100088. [CrossRef]
71. Pluskota, W.E.; Szablińska, J.; Obendorf, R.L.; Górecki, R.J.; Lahuta, L.B. Osmotic Stress Induces Genes, Enzymes and Accumulation of Galactinol, Raffinose and Stachyose in Seedlings of Pea (*Pisum sativum* L.). *Acta Physiol. Plant.* **2015**, *37*, 156. [CrossRef]
72. Sato, H.; Mizoi, J.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Complex Plant Responses to Drought and Heat Stress under Climate Change. *Plant J.* **2024**, *117*, 1873–1892. [CrossRef]
73. Liu, H.; Wang, F.; Liu, B.; Kong, F.; Fang, C. Significance of Raffinose Family Oligosaccharides (RFOs) Metabolism in Plants. *Adv. Biotechnol.* **2024**, *2*, 13. [CrossRef]

74. Nishizawa, A.; Yabuta, Y.; Shigeoka, S. Galactinol and Raffinose Constitute a Novel Function to Protect Plants from Oxidative Damage. *Plant Physiol.* **2008**, *147*, 1251–1263. [CrossRef]
75. Salvi, P.; Saxena, S.C.; Petla, B.P.; Kamble, N.U.; Kaur, H.; Verma, P.; Rao, V.; Ghosh, S.; Majee, M. Differentially Expressed Galactinol Synthase(s) in Chickpea Are Implicated in Seedvigor and Longevity by Limiting the Age Induced ROS Accumulation. *Sci. Rep.* **2016**, *6*, 35088. [CrossRef]
76. De Souza Vidigal, D.; Willems, L.; Van Arkel, J.; Dekkers, B.J.W.; Hilhorst, H.W.M.; Bentsink, L. Galactinol as Marker for Seed Longevity. *Plant Sci.* **2016**, *246*, 112–118. [CrossRef]
77. Vinson, C.C.; Mota, A.P.Z.; Porto, B.N.; Oliveira, T.N.; Sampaio, I.; Lacerda, A.L.; Danchin, E.G.J.; Guimaraes, P.M.; Williams, T.C.R.; Brasileiro, A.C.M. Characterization of Raffinose Metabolism Genes Uncovers a Wild Arachis Galactinol Synthase Conferring Tolerance to Abiotic Stresses. *Sci. Rep.* **2020**, *10*, 15258. [CrossRef]
78. De Koning, R.; Wils, G.E.; Kiekens, R.; De Vuyst, L.; Angenon, G. Impact of Drought and Salt Stress on Galactinol and Raffinose Family Oligosaccharides in Common Bean (*Phaseolus vulgaris*). *AoB Plants* **2023**, *15*, plad038. [CrossRef] [PubMed]
79. Dai, H.; Zhu, Z.; Wang, Z.; Zhang, Z.; Kong, W.; Miao, M. *Galactinol Synthase 1* Improves Cucumber Performance under Cold Stress by Enhancing Assimilate Translocation. *Hortic. Res.* **2022**, *9*, uhab063. [CrossRef] [PubMed]
80. Saxena, S.C.; Salvi, P.; Kaur, H.; Verma, P.; Petla, B.P.; Rao, V.; Kamble, N.; Majee, M. Differentially Expressed Myo-Inositol Monophosphatase Gene (*CaIMP*) in Chickpea (*Cicer arietinum* L.) Encodes a Lithium-Sensitive Phosphatase Enzyme with Broad Substrate Specificity and Improves Seed Germination and Seedling Growth under Abiotic Stresses. *J. Exp. Bot.* **2013**, *64*, 5623–5639. [CrossRef] [PubMed]
81. Idoudi, S.; Othman, K.B.; Bouajila, J.; Tourrette, A.; Romdhane, M.; Elfalleh, W. Influence of Extraction Techniques and Solvents on the Antioxidant and Biological Potential of Different Parts of Scorzonera Undulata. *Life* **2023**, *13*, 904. [CrossRef] [PubMed]
82. Nda-Umar, U.I.; Ramli, I.B.; Muhamad, E.N.; Azri, N.; Amadi, U.F.; Taufiq-Yap, Y.H. Influence of Heterogeneous Catalysts and Reaction Parameters on the Acetylation of Glycerol to Acetin: A Review. *Appl. Sci.* **2020**, *10*, 7155. [CrossRef]
83. Chen, T.; Mikhaylova, Y.V.; Shishova, M.F. Molecular Phylogenetic Analysis of the Tonoplast H+-ATPase Subunits. *Russ. J. Genet. Appl. Res.* **2017**, *7*, 592–606. [CrossRef]
84. Isayenkov, S.V. Plant Vacuoles: Physiological Roles and Mechanisms of Vacuolar Sorting and Vesicular Trafficking. *Cytol. Genet.* **2014**, *48*, 127–137. [CrossRef]
85. Cutzach, I.; Chatonnet, P.; Henry, R.; Dubourdieu, D. Identification of Volatile Compounds with a "Toasty" Aroma in Heated Oak Used in Barrelmaking. *J. Agric. Food Chem.* **1997**, *45*, 2217–2224. [CrossRef]
86. Takuli, P.; Khulbe, K.; Kumar, P.; Parki, A.; Syed, A.; Elgorban, A.M. Phytochemical Profiling, Antioxidant and Antibacterial Efficacy of a Native Himalayan Fern: Woodwardia Unigemmata (Makino) Nakai. *Saudi J. Biol. Sci.* **2020**, *27*, 1961–1967. [CrossRef]
87. Han, Y.; Xu, Q.; Hu, J.; Han, X.; Li, W.; Zhao, L. Maltol, a Food Flavoring Agent, Attenuates Acute Alcohol-Induced Oxidative Damage in Mice. *Nutrients* **2015**, *7*, 682–696. [CrossRef]
88. Yannai, S. (Ed.) *Dictionary of Food Compounds with CD-ROM*; Chapman and Hall/CRC: Boca Raton, FL, USA, 2003; ISBN 978-0-429-14923-8.
89. Bilova, T.; Greifenhagen, U.; Paudel, G.; Lukasheva, E.; Brauch, D.; Osmolovskaya, N.; Tarakhovskaya, E.; Balcke, G.U.; Tissier, A.; Vogt, T.; et al. Glycation of Plant Proteins under Environmental Stress—Methodological Approaches, Potential Mechanisms and Biological Role. In *Abiotic and Biotic Stress in Plants—Recent Advances and Future Perspectives*; Shanker, A.K., Shanker, C., Eds.; InTechOpen: London, UK, 2016; ISBN 978-953-51-2250-0.
90. Paudel, G.; Bilova, T.; Schmidt, R.; Greifenhagen, U.; Berger, R.; Tarakhovskaya, E.; Stöckhardt, S.; Balcke, G.U.; Humbeck, K.; Brandt, W.; et al. Osmotic Stress Is Accompanied by Protein Glycation in *Arabidopsis thaliana*. *J. Exp. Bot.* **2016**, *67*, 6283–6295. [CrossRef]
91. Shumilina, J.; Gorbach, D.; Popova, V.; Tsarev, A.; Kuznetsova, A.; Grashina, M.; Dorn, M.; Lukasheva, E.; Osmolovskaya, N.; Romanovskaya, E.; et al. Protein Glycation and Drought Response of Pea (*Pisum sativum* L.) Root Nodule Proteome: A Proteomics Approach. *Biol. Commun.* **2021**, *66*, 6283–6295. [CrossRef]
92. Rao, M.J.; Duan, M.; Zhou, C.; Jiao, J.; Cheng, P.; Yang, L.; Wei, W.; Shen, Q.; Ji, P.; Yang, Y.; et al. Antioxidant Defense System in Plants: Reactive Oxygen Species Production, Signaling, and Scavenging During Abiotic Stress-Induced Oxidative Damage. *Horticulturae* **2025**, *11*, 477. [CrossRef]
93. Shao, H.; Chu, L.; Shao, M.; Jaleel, C.A.; Hong-Mei, M. Higher Plant Antioxidants and Redox Signaling under Environmental Stresses. *C. R. Biol.* **2008**, *331*, 433–441. [CrossRef] [PubMed]
94. Krieger-Liszkay, A. Tocopherol Is the Scavenger of Singlet Oxygen Produced by the Triplet States of Chlorophyll in the PSII Reaction Centre. *J. Exp. Bot.* **2006**, *57*, 1677–1684. [CrossRef] [PubMed]
95. Shao, H.-B.; Chu, L.-Y.; Lu, Z.-H.; Kang, C.-M. Primary Antioxidant Free Radical Scavenging and Redox Signaling Pathways in Higher Plant Cells. *Int. J. Biol. Sci.* **2008**, *4*, 8–14. [CrossRef] [PubMed]
96. Spicher, L.; Almeida, J.; Gutbrod, K.; Pipitone, R.; Dörmann, P.; Glauser, G.; Rossi, M.; Kessler, F. Essential Role for Phytol Kinase and Tocopherol in Tolerance to Combined Light and Temperature Stress in Tomato. *J. Exp. Bot.* **2017**, *68*, 5845–5856. [CrossRef]

97. Mach, J. Phytol from Degradation of Chlorophyll Feeds Biosynthesis of Tocopherols. *Plant Cell* **2015**, *27*, tpc.15.00860. [CrossRef]
98. Smolikova, G.; Dolgikh, E.; Vikhnina, M.; Frolov, A.; Medvedev, S. Genetic and Hormonal Regulation of Chlorophyll Degradation during Maturation of Seeds with Green Embryos. *Int. J. Mol. Sci.* **2017**, *18*, 1993. [CrossRef]
99. Ahkami, A.H.; Wang, W.; Wietsma, T.W.; Winkler, T.; Lange, I.; Jansson, C.; Lange, B.M.; McDowell, N.G. Metabolic Shifts Associated with Drought-Induced Senescence in Brachypodium. *Plant Sci.* **2019**, *289*, 110278. [CrossRef]
100. Giner, R.M.; Ríos, J.L.; Máñez, S. Antioxidant Activity of Natural Hydroquinones. *Antioxidants* **2022**, *11*, 343. [CrossRef]
101. Dmitrieva, V.A.; Tyutereva, E.V.; Voitsekhovskaja, O.V. Singlet Oxygen in Plants: Generation, Detection, and Signaling Roles. *Int. J. Mol. Sci.* **2020**, *21*, 3237. [CrossRef]
102. Huang, S.; Van Aken, O.; Schwarzländer, M.; Belt, K.; Millar, A.H. The Roles of Mitochondrial Reactive Oxygen Species in Cellular Signaling and Stress Response in Plants. *Plant Physiol.* **2016**, *171*, 1551–1559. [CrossRef] [PubMed]
103. Mandal, M.; Sarkar, M.; Khan, A.; Biswas, M.; Masi, A.; Rakwal, R.; Agrawal, G.K.; Srivastava, A.; Sarkar, A. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) in Plants—Maintenance of Structural Individuality and Functional Blend. *Adv. Redox Res.* **2022**, *5*, 100039. [CrossRef]
104. Sood, M. Reactive Oxygen Species (ROS): Plant Perspectives on Oxidative Signalling and Biotic Stress Response. *Discov. Plants* **2025**, *2*, 187. [CrossRef]
105. Alché, J.D.D. A Concise Appraisal of Lipid Oxidation and Lipoxidation in Higher Plants. *Redox Biol.* **2019**, *23*, 101136. [CrossRef]
106. Chen, H.; Dong, J.; Wang, T. Autophagy in Plant Abiotic Stress Management. *Int. J. Mol. Sci.* **2021**, *22*, 4075. [CrossRef]
107. Tyutereva, E.V.; Murtuzova, A.V.; Voitsekhovskaja, O.V. Autophagy and the Energy Status of Plant Cells. *Russ. J. Plant Physiol.* **2022**, *69*, 19. [CrossRef]
108. Zharova, D.A.; Ivanova, A.N.; Drozdova, I.V.; Belyaeva, A.I.; Boldina, O.N.; Voitsekhovskaja, O.V.; Tyutereva, E.V. Role of Autophagy in Haematococcus Lacustris Cell Growth under Salinity. *Plants* **2022**, *11*, 197. [CrossRef]
109. Haghpanah, M.; Hashemipetroudi, S.; Arzani, A.; Araniti, F. Drought Tolerance in Plants: Physiological and Molecular Responses. *Plants* **2024**, *13*, 2962. [CrossRef]
110. Dutta, S.; Islam, Z.; Das, S.; Barman, A.; Chowdhury, M.; Mondal, B.P.; Ajnabi, J.; Manna, D. Harmonizing Plant Resilience: Unveiling the Symphony of Membrane Lipid Dynamics in Response to Abiotic Stresses: A Review. *Discov. Plants* **2025**, *2*, 61. [CrossRef]
111. Shajar, F.; Nabi, A.U.; Manzoor, A.; Saleem, S.; Mushtaq, N.U.; Manzoor, S.; Rehman, R.U. Membrane-Associated Mechanisms in Plant Responses to Abiotic Stress. *Plant Sci.* **2025**, *360*, 112726. [CrossRef]
112. Sah, S.K.; Fan, J.; Blanford, J.; Shanklin, J.; Xu, C. Physiological Functions of Phospholipid:Diacylglycerol Acyltransferases. *Plant Cell Physiol.* **2024**, *65*, 863–871. [CrossRef]
113. Bates, P.D.; Browse, J. The Significance of Different Diacylgycerol Synthesis Pathways on Plant Oil Composition and Bioengineering. *Front. Plant Sci.* **2012**, *3*, 29493. [CrossRef] [PubMed]
114. He, M.; Qin, C.-X.; Wang, X.; Ding, N.-Z. Plant Unsaturated Fatty Acids: Biosynthesis and Regulation. *Front. Plant Sci.* **2020**, *11*, 390. [CrossRef] [PubMed]
115. Sharma, P.; Lakra, N.; Goyal, A.; Ahlawat, Y.K.; Zaid, A.; Siddique, K.H.M. Drought and Heat Stress Mediated Activation of Lipid Signaling in Plants: A Critical Review. *Front. Plant Sci.* **2023**, *14*, 1216835. [CrossRef] [PubMed]
116. Hernández, M.L.; Cejudo, F.J. Chloroplast Lipids Metabolism and Function. A Redox Perspective. *Front. Plant Sci.* **2021**, *12*, 712022. [CrossRef]
117. Miège, C.; Maréchal, É. 1,2-Sn-Diacylglycerol in Plant Cells: Product, Substrate and Regulator. *Plant Physiol. Biochem.* **1999**, *37*, 795–808. [CrossRef]
118. Liu, H.-Z.; Li, Y.-K.; Chen, Y.-L.; Zhou, Y.; Sahu, S.K.; Liu, N.; Wu, H.; Shui, G.; Chen, Q.; Yao, N. Exploring the Plant Lipidome: Techniques, Challenges, and Prospects. *Adv. Biotechnol.* **2024**, *2*, 11. [CrossRef]
119. Dong, W.; Lv, H.; Xia, G.; Wang, M. Does Diacylglycerol Serve as a Signaling Molecule in Plants? *Plant Signal. Behav.* **2012**, *7*, 472–475. [CrossRef]
120. Zienkiewicz, K.; Ischebeck, T. Diacylglycerol in Plants: Functional Diversity of. In *Encyclopedia of Lipidomics*; Wenk, M.R., Ed.; Springer: Dordrecht, The Netherlands, 2017; pp. 1–4. ISBN 978-94-007-7864-1.
121. Rogowska, A.; Szakiel, A. The Role of Sterols in Plant Response to Abiotic Stress. *Phytochem. Rev.* **2020**, *19*, 1525–1538. [CrossRef]
122. Du, Y.; Fu, X.; Chu, Y.; Wu, P.; Liu, Y.; Ma, L.; Tian, H.; Zhu, B. Biosynthesis and the Roles of Plant Sterols in Development and Stress Responses. *Int. J. Mol. Sci.* **2022**, *23*, 2332. [CrossRef]
123. Hadacek, F. Sterol Lipids in Plants: Functional Diversity of. In *Encyclopedia of Lipidomics*; Wenk, M.R., Ed.; Springer: Dordrecht, The Netherlands, 2017; pp. 1–4. ISBN 978-94-007-7864-1.
124. Hartmann, M. Plant Sterols and the Membrane Environment. *Trends Plant Sci.* **1998**, *3*, 170–175. [CrossRef]
125. Puzanskiy, R.K.; Kirpichnikova, A.A.; Bogdanova, E.M.; Prokopiev, I.A.; Shavarda, A.L.; Romanyuk, D.A.; Vanisov, S.A.; Yemelyanov, V.V.; Shishova, M.F. From Division to Death: Metabolomic Analysis of Nicotiana Tabacum BY-2 Cells Reveals the Complexity of Life in Batch Culture. *Plants* **2024**, *13*, 3426. [CrossRef] [PubMed]

126. Zaaboul, F.; Liu, Y. Vitamin E in Foodstuff: Nutritional, Analytical, and Food Technology Aspects. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 964–998. [CrossRef] [PubMed]
127. Bobe, G.; Zhang, Z.; Kopp, R.; Garzotto, M.; Shannon, J.; Takata, Y. Phytol and Its Metabolites Phytanic and Pristanic Acids for Risk of Cancer: Current Evidence and Future Directions. *Eur. J. Cancer Prev.* **2020**, *29*, 191–200. [CrossRef]
128. Mudgil, D.; Barak, S.; Khatkar, B.S. Guar Gum: Processing, Properties and Food Applications—A Review. *J. Food Sci. Technol.* **2014**, *51*, 409–418. [CrossRef]
129. Kose, F.; Weckwerth, W.; Linke, T.; Fiehn, O. Visualizing Plant Metabolomic Correlation Networks Using Clique–Metabolite Matrices. *Bioinformatics* **2001**, *17*, 1198–1208. [CrossRef]
130. Fukushima, A.; Kusano, M.; Redestig, H.; Arita, M.; Saito, K. Metabolomic Correlation-Network Modules in Arabidopsis Based on a Graph-Clustering Approach. *BMC Syst. Biol.* **2011**, *5*, 1. [CrossRef]
131. Szymanski, J.; Jozefczuk, S.; Nikoloski, Z.; Selbig, J.; Nikiforova, V.; Catchpole, G.; Willmitzer, L. Stability of Metabolic Correlations under Changing Environmental Conditions in Escherichia Coli—A Systems Approach. *PLoS ONE* **2009**, *4*, e7441. [CrossRef]
132. Kotze, H.L.; Armitage, E.G.; Sharkey, K.J.; Allwood, J.W.; Dunn, W.B.; Williams, K.J.; Goodacre, R. A Novel Untargeted Metabolomics Correlation-Based Network Analysis Incorporating Human Metabolic Reconstructions. *BMC Syst. Biol.* **2013**, *7*, 107. [CrossRef]
133. Camacho, D.; De La Fuente, A.; Mendes, P. The Origin of Correlations in Metabolomics Data. *Metabolomics* **2005**, *1*, 53–63. [CrossRef]
134. Steuer, R. Review: On the Analysis and Interpretation of Correlations in Metabolomic Data. *Brief. Bioinform.* **2006**, *7*, 151–158. [CrossRef]
135. Rosato, A.; Tenori, L.; Cascante, M.; De Atauri Carulla, P.R.; Martins Dos Santos, V.A.P.; Saccenti, E. From Correlation to Causation: Analysis of Metabolomics Data Using Systems Biology Approaches. *Metabolomics* **2018**, *14*, 37. [CrossRef]

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