Assessment of phenotypic trait plasticity in the oilseed *Camelina sativa* using integrated early-stage abiotic stress and field studies

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Running Title: Plasticity of *Camelina sativa* to abiotic stress

Abstract

Connecting the characterisation of juvenile (pre-anthesis) plant stress responses in controlled environments to field agronomic performance is a challenge. The oilseed crop *Camelina sativa* (camelina), with its innate resilience and plasticity, presents an opportunity to understand both the underlying mechanisms of juvenile resilience and identify the implications for yield in diverse pedoclimates. A better understanding of camelina's abiotic stress resilience is important in the context of climate change and the development of breeding programmes for climate-tolerant crops. In this study, 54 accessions representing the genetic diversity observed in the wider publicly available population were used to investigate the plasticity of camelina's early-stage response to drought and heat stress, combined with an evaluation of field performance in multi-location field trials. A combinatorial phenotyping approach of early-stage drought and heat stress identified stress-responsive signatures within the diversity panel. The substantial variation in the morphophysiological line-specific responses to stress, indicated that juvenile and mature camelina plants have significant plasticity and access different stress response strategies. In response to stress, we observed significant molecular metabolic adjustment alongside significant lipid remodelling and physiological compensation. Camelina was resilient to drought stress, and certain metabolites were identified as indicators of abiotic stress response. Applying an integrated approach, early-stage phenotyping and multi-location field trials provided a complete assessment of the camelina stress response and facilitated a connection to crop productivity. This approach facilitates improved breeding programmes, addresses the restrictions of limited genetic diversity in camelina, and supports the development of local varieties optimised for climate resilience.

Introduction

Agriculture faces increasing threats from extreme weather events associated with anthropogenic climate change (Ray et al. 2015; Lesk et al. 2016). Fluctuating hot and dry conditions associated with elevated temperatures and irregular precipitation pose a particular risk to major staple crops (Lesk et al. 2021) and often result in significant yield reduction. Heat and drought stress restrict germination, and impair development, growth, and reproduction. Temperate Brassica oilseeds, like oilseed rape (*Brassica napus*), are particularly sensitive to increasing temperatures and water limitation (Secchi et al. 2023). Research has established a negative relationship between abiotic (heat and drought) stress and agronomic performance e.g., seed yield and quality (Jensen et al. 1996; Yu et al. 2014), although the impact of abiotic stress on seed oil biosynthesis and regulation is still uncertain.

Diversifying cropping systems with better-adapted oilseeds could potentially stabilise productivity under changing environments associated with climate change. Growers are returning to traditional oilseeds like *Camelina sativa* L. Crantz (camelina), a *Brassicaceae* crop with a recognised commercial value that results from its seed oil quality i.e., elevated levels of omega-3 α-linolenic acid, which is nutritionally important. Beyond nutrition, camelina seed oil is widely used, e.g., food, biofuel, and feed stock for biobased industries. Moreover, camelina has resilience to challenging environments and can be utilised in different cropping systems. Its two crop biotypes, spring and winter (Zubr, 1997), and suitability for different pedoclimates further expand its cultivation area and utilisation. Furthermore, the low agricultural input requirement (fertilisation), and short growing cycle make camelina a good candidate for expansion into marginal land not commercially cultivated (Zanetti et al. 2021). As an ancestral crop, camelina genetic diversity is only now receiving attention (Luo et al. 2019, Decker et al. 2025). Studies have focused on genomic and seed traits among natural accessions and breeding lines, to understand the genetic mechanisms underlying productivity (Li et al. 2021; Hotton et al. 2020; King et al. 2019; Vollmann et al. 2007). Recognised for its tolerance to biotic and abiotic stresses, camelina is a good model to study plasticity (Großkinsky et al. 2023). In comparison with oilseed rape, Gao et al. (2018) reported that camelina showed more tolerance to drought stress. However, the ability of camelina to tolerate extreme drought stress varies with genotype (Čanak et al. 2020) indicating untapped potential. Camelina accessions and cultivars grow in varying climatic niches throughout the globe, and temperature was found to elicit plasticity in camelina seed oil (Brock et al. 2020). The impact of post-anthesis heat stress on oil yield and fatty acid composition in camelina has been established, reducing seed yield, weight, oil content and altering fatty acid composition (Nadakuduti et al. 2023; Smith and Lu 2024). Several approaches, such as quantitative trait locus (QTL) mapping, genome wide association, and comparative transcriptome profiling have also begun to characterise the pathways controlling heat tolerance during reproduction (Smith et al. 2024). In the field, abiotic stress can affect plants at any point during development. In young tissues under abiotic stress, there is evidence for higher plasticity in traits associated with growth responses (reviewed in Rankenberg et al. 2021). However, experimental evidence and understanding of early-stage abiotic stress responses are limited. Despite the recognised abiotic stress tolerance and plasticity of camelina, no studies have investigated how it responds to heat and drought in early growth stages. To address this complexity, metabolomic and biochemical profiling can now tell us about the biology of the plant response to abiotic stress and support genomic selection, by identifying traits of interest (Fernandez et al. 2021; Alwood et al. 2021).

Effective solutions to develop stress-tolerant crops require the inclusion of realistic agricultural field studies in fundamental research (Prado et al. 2025). To address this challenge, we investigated the physiological and molecular mechanisms underlying stress resilience using a combinatorial approach in multiple settings. Improving tolerance to elevated temperatures and drought is essential for camelina agronomic sustainability in growing seasons with increasing meteorological instability. The objectives of this study were to increase our understanding of the camelina multilevel abiotic stress response, characterise the plasticity of this response in different lines and establish how the diversity of stress responses translates into improved agronomic performance in the field. To achieve this, a combinatorial approach was used to monitor dynamic changes in morphological, physiological, and metabolic responses of a camelina diversity collection to early-stage heat and drought stress. In parallel, the collection was grown in multi-location field trials (UK, Italy and France) with diverse environments providing real-world heat and drought conditions, enabling a fully integrated evaluation of the stress response in camelina, plasticity and connection to agronomic performance. These results offer diagnostic markers and a framework to assess crop stress resilience, advancing the development of climate-resilient oilseeds and facilitating the integration of genetic diversity into cropping systems.

Results

Genetic diversity and population structure in the camelina study panel

A total of 54 spring-type camelina accessions were selected to represent commercial and local varieties spanning diverse pedoclimates. To determine how representative the 54 accessions in the study panel (henceforth, UNT lines, Supplementary Table S1) were of the available public accessions, a total of 200 camelina accessions (Li et al. 2021) were analysed using two approaches. Firstly, sequencing reads were aligned to the camelina DH55 reference genome (Kagale et al. 2014) to identify a single nucleotide polymorphism (SNP) variant matrix of 340,696 high-confidence SNPs. A Principal Component Analysis (PCA) of this matrix (Fig. 1A) indicated that the study panel was representative of the wider population, with PC 1 and 2 explaining 21% and 12% of the genetic variance, respectively, indicating a good representation of the total genomic diversity space. Secondly, to elucidate the pairwise genomic similarity in detail, a hierarchical clustering based on Identity-By-State distances was generated aligning the resulting dendrogram tree with the results from an ADMIXTURE population genetics analysis (Fig. 1B). In this analysis, the public (Li et al. 2021) and study panel camelina accessions were grouped by their genomic relatedness to infer which fractions of their genomes, based on SNP data, belong to which ancestral population. The ADMIXTURE analysis performed most effectively with eight assumed subpopulations (Fig. 1B). Collectively this indicated that the study panel captured most of the genetic diversity observed in the larger public population and provided a representative resource to phenotype camelina morphophysiological responses to abiotic stress.

Metabolic plasticity of the camelina panel to abiotic stress

To assess the response to abiotic stress, early-stage drought and heat were imposed separately on 18-day-old plantlets for up to ten days (Supplementary Fig. S1). Drought was imposed by water withdrawal to 20% soil water content (SWC) and monitored thereafter (Supplementary Fig. S1 A). High temperature (32/25°C day/night) was tracked using growing degree days (GDD, Supplementary Fig. S1 B). Plant development and growth were assessed by development stage 1 of BBCH scale (Martinelli and Galasso 2011), leaf length, and width (Supplementary Fig. S2). All three traits demonstrated highly significant differences with treatment and line (Supplementary Table S2 A, B, C). Overall, heat stress advanced development, whereas drought stress had the opposite effect, together with significantly reduced leaf length and width. These results suggested that the imposed conditions caused a significant change in camelina development and merited further investigation of the plant's metabolism.

To do this, an (un)targeted metabolomics approach using complementary techniques was employed to profile both primary and secondary leaf metabolites in response to the applied stresses. A PCA of the targeted (ten major) biomass compounds evaluated the global impact of drought and heat on camelina biomass (Fig. 2A). The PC1 vs PC2 score plot explained 70.4% of the total variance, showing that while ambient and heat stress conditions partially overlapped, drought stress was more scattered and had a more substantial impact, corresponding to separation along PC 1. This indicated that camelina's central metabolism responded differently to each stress, with drought having a more substantial effect at early-stage than heat. The heatmap clustering analysis (Fig. 2B) visualised relationships between samples and major compounds: all ten biomass compound variables were statistically significant with respect to growth conditions (ANOVA, $P < 0.01$, FDR). Consistent with the PCA plot in Fig. 2A, drought stress clustered separately from ambient and heat stress conditions. Most of the major compounds analysed accumulated under drought, except citrate and starch. Heat stress generally decreased compound content, except for starch, which increased, and soluble sugars (e.g., glucose, sucrose), which remained stable.

Untargeted metabolomics further revealed clear shifts in the camelina metabolome between drought and heat stress (Fig. 2C; Supplementary Fig. S3). The analysis identified 3016 metabolomic features, of which 1350 were unknowns, 1446 had suggested annotated metabolites and 220 matched with identified metabolites (Supplementary Fig. S3 A). Among these 3016 features, which were normalised prior to statistical analyses, 2545 were statistically significant regarding growth conditions (ANOVA $P < 0.01$, FDR, Supplementary Fig. S3), indicating the robustness of the metabolomic dataset and the level of phytochemical diversity shifts. The global impact of drought and heat on camelina phytochemical diversity was assessed by PCA (Fig. 2C), where PC1 vs PC2 separated control, drought and heat stress with a total variance of 30.3%. This indicated that the global metabolic profiles of camelina responded differentially to the type of stress. Since this metabolomic profile is mostly composed of secondary plant molecules, it further identified that stress-responsive, secondary metabolism is a better integrator to distinguish drought and heat impact than major compounds (Fig. 2A). In response to drought, 308 metabolic markers accumulated and 283 decreased, while in response to heat stress, 327 increased and 271 were depleted (Fig. 2 D, E; Supplementary Fig. S3 B, FC = 2, *t*-test $P < 0.01$, FDR, between control and stress). For each stress, a balanced number of specific (128 and 147 for drought and heat respectively) and common (180) accumulated markers and specific (164 and 152 for drought and heat respectively) and common (119) depleted markers were observed. The accumulated and depleted markers for drought and heat stress were then stacked for each camelina line in the diversity panel (Fig. 3). This approach uncovered clear differences in the metabolic response of the different camelina lines to drought and heat stress. Some lines showed no or very low numbers of responding metabolic markers (e.g., UNT7, 44, and 45 under drought - Fig. 3A; UNT10, 14 and 29 under heat - Fig. 3B). Other lines showed a pronounced overall metabolic response with high number of accumulated/depleted markers, e.g., UNT16, 33, 57, and 59 in drought (Fig. 3A), and UNT5, 18, 32, 38 and 50 in heat (Fig. 3B). Furthermore, some camelina lines had a strong response to drought but much less to heat (e.g., UNT16) and conversely individual lines had a strong response to heat and much less to drought (e.g., UNT5), whereas other lines (e.g., UNT33) had a large metabolic response to both drought and heat. Thus, adjustment of metabolism appeared to be an important part of the response of camelina to drought and heat stress, and there was substantial variation among lines suggesting plasticity in the camelina metabolic response to specific stresses.

To gain further metabolic insight, metabolic markers were putatively annotated using ClassyFire ontologies (Djoumbou Feunang et al. 2016). Common markers (Table 1) included some increased phenylpropanoids (cinnamic and coumaric acids, flavonoids), carbohydrates (pentoses and phenolic glycosides), and benzenoids, along with several phenylpropanoids (phenolic acids and flavonoids) and organic acids (citric acid). Under drought, responses were diverse, with increased phenylpropanoids, phenolic acids and anthocyanins, amino acids (proline, histidine, and tyrosine derivatives), carbohydrates (phenolic glycosides), and lipids (terpene glycosides), as well as some decreased phenylpropanoids (chalcones and flavonoid glycosides), organic acids (succinic acid), benzenoids (benzoic acid derivatives), nucleotides, and lipids (fatty acids). Heat stress positively impacted phenylpropanoids (chalcones, flavans, and flavonoid glycosides), terpenes, benzenoids, and carbohydrates (O-glycosyl compounds), while negatively affecting some other phenylpropanoids (flavonoids, stilbenes, and phenolic acids), organic acids (TCA derivatives), lignans, and benzenoids (benzoic acids). Overall, metabolomics revealed a profound modification of primary (i.e. amino and organic acids, lipids) and secondary (i.e. phenylpropanoids, terpenes) metabolisms in responses to stress, with well-established markers.

Camelina fatty acid desaturation and remodelling in response to abiotic stress

Leaf fatty acids (FA) from the camelina early-stage drought and heat stress experiment showed a significant impact of stress on total FA, relative polyunsaturated FA content (PUFA index) and C18:3 content (linolenic acid, Fig. 4 A, C, D; Supplementary Table S2 D, E, F). C18:0 demonstrated a highly significant line effect and marginally significant effect of the stress (Supplementary Table S2 G), whilst C18:3 exhibited significant effects due to environmental stress and only marginal line differences. Lipid remodelling clearly demonstrated an impact of abiotic stress, and a response was observed across all accessions. The PUFA index reflected FA desaturation adjustment with heat as camelina remodelled membrane FA composition to maintain its fluidity. Heat decreased the calculated PUFA index to 2.2 compared to 2.8 under ambient conditions, whilst drought had the largest effect on total FA content, increasing the average amount to 182.4 µg/mg compared to 98.8 µg/mg under ambient. Plotting total FA and PUFA index (Fig. 4 A) for each line clearly separated the two stress treatments and ambient. Oleic acid (C18:1) significantly declined with drought and increased with heat in all lines (Fig. 4 C) and was the best indicator of environmental stress response in camelina. Within the population there was a line-specific response, with some lines showing significant changes with both treatments e.g., UNT 5, 21, 31, 33, 38 and 58, whilst others had a stress-specific response e.g., UNT22 and 37 – responding to heat but not drought (Fig. 4 C). Camelina remodelled its FA composition and content in response to environmental stress, and the extent of this remodelling was accession specific within the panel.

To further establish if leaf FA remodelling in response to abiotic stress reflected the response of mature plants in agronomic field trials, FA were analysed in mature seeds collected from the diversity collection grown in multi-location field trials (Fig. 4 B, E; Table 2; Supplementary Table S3). The three trials (France, Italy and UK; see supplementary methods) represented different pedoclimates and environmental challenges, namely climate during seed maturation i.e., from 50% flowering to harvest and summarised as: Italy – drought (GDD 828, precipitation 51.8mm); France - heat (GDD 1235, precipitation 224mm); and UK - temperate (GDD 721, precipitation 137mm). For reference, long term annual averages for precipitation and temperature at the trial locations are: UK precipitation 702mm, temperature 10.3°C; France precipitation 772mm, temperature 11.1°C; and Italy 613mm precipitation, 13.2℃ (details in Supplementary Methods). Camelina seed lipid metabolism responded to environmental stress by remodelling FA, as indicated by the separation of Italy (drought) and France (heat) from the UK in the plot of total fatty acids and relative PUFA content (Fig. 4 B); changing the levels of desaturation (Fig 4 E) at each trial location. Collectively, the controlled environment and multilocation field trials showed how camelina effectively utilised lipid metabolism and remodelling to respond to abiotic stress, and therefore, how FA composition can be used to identify stress responsive lines.

Integrative analysis of the morphophysiological and metabolic response of camelina to abiotic stress

The impact of early-stage abiotic stress in leaves was comprehensively assessed by measuring plant water content (PWC), $\Delta^{15}N$ and $\Delta^{13}C$ isotope discrimination, total antioxidant capacity (TAC), and relative chlorophyll content (SPAD, Supplementary Fig. S4, S5). On average, TAC decreased under both stress conditions, suggesting oxidative stress, with heat resulting in a stronger effect. Significant differences in PWC, Δ^{13}C isotope discrimination and TAC were observed with respect to both treatment and line (Supplementary Table S2 H, I, J). Furthermore, leaf Δ^{15}N significantly responded to heat treatment (Supplementary Fig. S4 B; Supplementary Table S2 K).

Overall, heat stress advanced development whereas drought stress had the opposite effect, significantly reducing leaf length and width. Individual lines showed differing responses to abiotic stress (Supplementary Fig. S2), for example lines UNT11, 12, 18, 21, 32, 55 showed only a limited developmental response to stress, whilst others (e.g., UNT6, 8, 34, 56) had a much greater response. Drought stress had a significant impact on plant water content (PWC, Supplementary Table S2 H, Supplementary Fig. S4 C). Individual lines lowered their PWC to less than 80% e.g., UNT10, 11, 15, 57, whereas others maintained PWC at control levels (87%) despite the drought treatment e.g., UNT35, 44, 53, 54 (Supplementary Fig. S4 C). PWC was maintained in heat stress plants, indicating that combined heat and drought stress did not occur in this experiment. As a further proxy measure of water availability, the degree of Δ^{13}C isotope discrimination was determined in leaf samples (Supplementary Fig. S4 A). Drought resulted in a reduction in Δ^{13}C in the study panel, which was significant for both treatment and line (*P* <0.0001; Supplementary Table S2 I). Both the measurement of PWC and Δ^{13}C indicated that the camelina study panel contained lines with variability in drought tolerance (Supplementary Fig. S4). Resistance to environmental stress was also evaluated by measuring leaf chlorophyll content (SPAD) as an indicator of stress tolerance. The average SPAD measurements for the diversity panel grown in ambient conditions was 35.7 Chlorophyll index (CI) units, heat 36.65 CI units, and drought 44.09 CI units (Supplementary Fig. S5). These measurements indicated an increase in chlorophyll content following drought. However, not all the lines responded in the same way. For example, at the end of the experiment, UNT5 and 12 had the highest CI values under ambient conditions, but UNT7 and 29 ranked highest following drought, whilst UNT12 and 25 ranked higher after heat. UNT31 consistently had the lowest CI value in ambient, but under drought, UNT46 was lowest, and in heat UNT55. These results identified that chlorophyll content was differentially modulated in camelina lines to maintain photosynthetic performance under abiotic stress.

Cellular redox balance is crucial for abiotic stress response and acclimation. Metabolomics identified genotype-specific differences in antioxidants, and estimations of the redox balancing capacity of the studied camelina lines were made, determined as TAC in leaves (Supplementary Fig. S4 D). On average, TAC decreased under both stress conditions in the diversity panel suggesting oxidative stress, with heat resulting in lower TAC. Line-specific responses were highly significant effect (P<0.0001, Supplementary Table S2 J). Under ambient conditions UNT28 exhibited the highest capacity and UNT45 the lowest, however UNT8 and 25 showed a higher overall TAC under drought. Some of the lines showed reduced TAC under both stresses, e.g., UNT11, 40, and 58; whilst others e.g., UNT13, 56, 39, and 22 only responded significantly to drought; and some e.g., UNT4, 14, 16, 54 only to heat, indicating that the study panel had significant variability, in terms of cellular redox response, to abiotic stress.

The collected data traits, i.e. morphophysiological markers comprising, TAC, leaf FA, SPAD, stable isotope discrimination, phenology (BBCH), plant development (stem thickness, leaf width, number and length, SWC, PWC, and plant fresh and dry weight), were combined with FA and targeted metabolites for an integrated analysis by PCA for drought and heat stress (Fig. 5). Camelina showed a clear developmental, physiological, and biochemical response to abiotic stress. In response to heat and drought, plant development and FA contributed strongly to this analysis (Fig. 5B and D, illustrated by red and orange arrows). It was interesting to note that Δ^{13}C discrimination had a larger impact in heat than drought, despite SWC and PWC being maintained at ambient levels in heat stress. However, the line distributions were different in response to each stress (Fig. 5A and C). Metabolic, biochemical and physiological parameters (e.g., citrate, malate, polyphenols, chlorophyll, TAC, stable isotope discrimination and plant water status) identified 'responsive' lines e.g., UNT5, 18, 22, 28, 31, 35, 59 (red) and 'unresponsive' e.g., UNT lines 1, 8, 13, 34, 50, 52 (blue; Fig. 5A; Supplementary Table S4 A) to drought. The distribution of lines in heat showed responsive, e.g., UNT18, 31, 50, 56 (red/orange) and unresponsive lines e.g., UNT3, 8, 9, 13, 38, 52 (blue Fig. 5C; Supplementary Table S4 B). Abiotic stress-responsive lines common to heat and drought included UNT18 and 31, whilst common unresponsive lines were UNT8, 13, and 52. However, there was little commonality in these lines in terms of accumulation/depletion of metabolic markers in response to stress (Fig. 3) or genetics (Fig. 1).

Connecting plasticity in camelina stress responses to agronomic performance

Multi-location field trials in diverse pedoclimates were used to assess the response of mature plants to challenging environments and assess the agronomic performance of the camelina panel. Yield, plant density, stand height, thousand grain weight (TGW), and seed oil content/composition were measured (Table 2, Supplementary Fig. S6, S7; Supplementary Table S3, S5) in the diversity panel at the three locations: Italy – drought; France - heat; and UK - temperate. Seed oil content and composition, together with TGW across lines remained relatively consistent in all three field trial locations, whereas other phenotypic traits (yield, height, density) were clearly different (Supplementary Fig. S6, S7).

Overall, the diversity panel had the highest yield in Italy; the camelina lines responding well to drought, whereas in France (heat) the lines grew taller, accumulating biomass with the increase in temperature and sufficient precipitation. In response to the different environments the coefficient of variation (CV; Supplementary Table S6) estimated for TGW, oil content and yield at the three locations indicated limited variation for oil content (4.4 to 5.1) and TGW (12.5 to 13.9), whilst the panel CV for yield was high and more variable (22.4 to 57.2). Most notably, yield in Italy (drought) appeared to be much more robust amongst the lines and demonstrated a different relationship to other traits when compared to France (heat) and UK (temperate) (Supplementary Fig. S6 F). Assessment of agronomic performance distinguished individual lines in their response to the local climates (Supplementary Table S4, Supplementary Fig. S6). UNT57 had the highest TGW at all three trial locations, whereas UNT33, 21, and 59 had the lowest. As indicated by the CV (Supplementary Table S6), TGW was stable across environments, while yield and seed oil content were more variable and sensitive to genotypic x environment (G x E) interactions at all locations. Analysis of mature seed FA content showed a strong correlation in composition across the three trial locations (Supplementary Fig. S7). Genotypic trends in FA composition were relatively consistent across the three trials. In particular, the panel lines aligned similarly in the first two PC projections across the trial locations. PCA analysis of seed FA (Supplementary Fig. S7 B) identified responsive (e.g., UNT12 15, 19, 21, 22, and 55) and unresponsive (e.g., UNT46, and 54) lines consistently across the three locations. Location-specific differences in PC1 appeared to be driven by C20:1 (UK – temperate different to France and Italy – abiotic stress) and C20:0 (France (heat) in contrast to the UK (temperate) and Italy (heat)).

As discussed above, the studied lines showed significant differences in their metabolic, physiological and developmental responses to heat and drought. Supplementary Table S4 illustrates the different stress-responsive strategies deployed by these lines, e.g., in drought UNT31 was highly responsive in the combined PCA analysis but had a low ranking in accumulated/depleted markers, whereas UNT22 was also responsive but accumulated/depleted substantial numbers (~600) of metabolic markers (Supplementary Table S4 A). The distribution for unresponsive (Fig.6) lines was similar, with individual lines e.g., UNT50 and 31, showing a range of accumulated/depleted metabolic markers. The pattern was repeated for heat stress (Supplementary Table S4 B). PCA analysis identified unresponsive e.g., UNT8 and 13, and responsive lines e.g., UNT56 and 18 with divergent metabolic marker responses. Our approaches provided a unique opportunity to evaluate how these differing response patterns could be related to agronomic performance. Looking closely at the stress (un)responsive subset, lines UNT22 and 59 were responsive to drought (PCA) with large numbers of accumulated/depleted metabolic markers and had a very low

TGW across all three trials. In this case, UNT22 and 59 showed a strong stress response which did not translate into good field performance. In contrast, UNT5 and 28 were also responsive to drought (PCA) but did not accumulate/deplete large numbers of metabolic markers and yet performed well in the field. This case illustrates how lines UNT5 and 28 maintained field performance via physiological adaptation rather than metabolic adjustment (as determined by metabolic profiling). Field performance (TGW) differentiated lines unresponsive to drought (PCA), and with a similar metabolic marker responsiveness e.g., UNT34 and 13. Indeed, UNT8 performed very well in the field (TGW and oil content), was unresponsive (Fig 6) and had a moderate metabolic marker drought response. Metabolic responses in UNT8 might have enabled the plant to prosper in response to both drought and heat. Heat stress produced a similar plasticity of responses across the measured parameters for early-stage stress (PCA and metabolic markers; Supplementary Table S4 B). Some lines performed well in the field (TGW) e.g., UNT8 and 13, and were unresponsive to heat (PCA), whereas others were responsive to heat and performed badly in the field e.g., UNT31 and 50. These results indicate that crop agronomic performance was not necessarily associated with robust metabolism and therefore low plasticity. On the contrary, high plant plasticity with dynamic metabolism could therefore be coupled with improved agronomic performance, identifying metabolic plasticity as an indicator of resilience to abiotic stress and field crop performance in changing climates. Further analysis was performed to establish a relationship between the metabolic clustering presented in each of the early-stage conditions applied, control, drought stress and heat stress, and three main measures of agronomic performance, TGW, seed yield and oil content (Fig. 6). However, global metabolomic performance in each of the conditions applied did not correlate with agronomic performance (Fig. 6 A). To evaluate the concordance between genotype and phenotype-related data, Baker's Gamma correlation coefficients were computed between the dendrograms obtained from genomic, agronomic, and condition-specific metabolomic data (Fig. 6 B). The highest correlation was observed between the genomic dendrogram and the metabolomic clustering under drought stress (0.52), indicating a moderate structural similarity and the possibility that camelina is inherently adapted to drought. A weaker, but still positive, correlation was also found between genomic and agronomic clustering (0.23). The other comparisons showed lower correlations: between metabolomics under drought and heat stress (0.17), between heat stress metabolomics and genomics (0.15), between agronomic and drought metabolomics (0.12), and finally between metabolomics under heat and control conditions (0.08).

Discussion

Camelina genetic diversity and the potential for crop improvement

An understanding of how available genetic diversity in camelina underpins its stress resilience is vital for developing a location-specific breeding strategy for further crop improvement and to understand the plasticity of abiotic stress responses. Camelina (Brassicaceae) has a hexaploid genome structure generated by the merger of three diploid genomes (Mandáková et al. 2019; Mandáková and Lysak 2022; Bird et al. 2024). Reports in the literature have generally suggested low levels of genetic diversity in camelina compared to other oilseed crops (Chaudhary et al. 2020). This genetic paucity has been recognised with approaches developed to address the diversity challenge (Blume et al. 2023). However, it is noteworthy that detailed explorations of large camelina populations (see Luo et al. 2019) have demonstrated enough genetic diversity for developing new cultivars with desirable agronomic traits. Moreover, abiotic stress studies (Čanak et al. 2020; Smith and Lu 2024) have shown diverse responses of different camelina genotypes. However, much of camelina's varietal diversity has been lost, particularly when European farmers shifted their interest from camelina to rapeseed and sunflower. Therefore, current publicly available germplasm collections are almost entirely composed of previous cultivated varieties and have a low genetic diversity and a high proportion of admixture. Many camelina breeding lines and cultivars were collected from the Russia-Ukraine region (Vollmann et al. 2007), the common origin area of camelina (Brock et al. 2018). In this study, we demonstrated that the collected camelina panel adequately represented the publicly available camelina germplasm. Furthermore, sequencing analysis identified the presence of eight sub-populations, and PCA based on SNPs revealed that the study panel provided a good representation of the global camelina genomic diversity space. Knowledge of population structure and genetic diversity within this panel enabled a detailed investigation of camelina genotype-specific abiotic stress responses.

Camelina abiotic stress metabolic response

The imposition of heat and drought stress on the camelina panel at a juvenile stage of development had a significant impact on development and growth (Fig. 7). Plants were smaller and grew slowly with drought, whilst heat accelerated the developmental stages. These responses significantly differed between lines, indicating that the existing genetic diversity of camelina supports a portfolio of morphophysiological adaptation strategies. Although responses of this type might be anticipated to heat and drought stress (Čanak et al. 2020; Nadakuduti et al. 2023; Smith et al. 2024), this study demonstrated the impact of abiotic stress at the juvenile stage across a diversity panel and the translation of this robustness to different field environments. Developmental age is a strong determinant of stress responses in plants (Rankenberg et al. 2021). With aging, plants alter their organ morphology, sink–source balance, and chemical composition, including changes in redox status, which collectively influence how abiotic stress signals are perceived and processed. In this study, initial investigations focused on the physiological and metabolic response to drought and heat stress in young leaves before the initiation of inflorescences to understand the plasticity of stress resilience early in juvenile development. Drought had a significant impact on plant water relations and gas exchange. The reduced level of carbon isotope fractionation ($\Delta^{13}C$) discrimination was consistent with crop drought responses (Avramova et al. 2019). Plant $\Delta^{15}N$ reflects the values of external N sources and $^{15}N/^{14}N$ fractionations which occur during assimilation, transport and loss of N (Robinson et al. 2001). $\Delta^{13}C$ and $\Delta^{15}N$ are often used to determine drought responses in crops, and as expected, $\Delta^{13}C$ was a good indicator of drought stress (reflecting changes in stomatal opening), whilst $\Delta^{15}N$ separated ambient and heat stressed plants. Heat stress impacted growth and development, leaf nutrient status, uptake and translocation (Mishra et al. 2023). Likewise, TAC in young camelina leaves varied significantly and was typically reduced by heat and drought. Juvenile camelina plants demonstrated significant plasticity in response to abiotic stress. A significant part of this response relied on metabolic adjustment, likely triggered by a network of signalling cascades, mediated by ROS (Choudhury et al. 2017) and lipid signals (Sharma et al. 2023).

Metabolic adjustment was central to the abiotic stress tolerance and plasticity observed. Targeted leaf metabolite analysis of juvenile leaves identified an asymmetric response to heat and drought. For example, citrate, malate, sucrose, glucose, proteins and amino acids declined with heat and increased with drought, whereas starch increased with heat and declined with drought. Typically, abiotic stress impacts growth earlier than photosynthesis, which leads to energy imbalance and favours the accumulation of ROS. Plants respond by shifting to protective mechanisms, which include the accumulation of various metabolites (Zandalinas et al. 2022), including antioxidants (Decros et al. 2019). Major carbohydrates such as glucose, fructose, and sucrose have been shown to accumulate and starch to decrease in many species in response to drought (reviewed in Zandalinas et al. 2022), reflecting reduced plant growth (decreasing the demand for primary metabolites) combined with a shift towards soluble compounds contributing to osmotic adjustment (Hummel et al. 2010; Hildebrandt 2018, Heinemann and Hildebrandt 2021). Heat led to a reduction in levels of primary metabolites in camelina, a response that reflected the effect of heat on carbon utilization and distribution in plants. Together, photosynthesis and respiration underpin the plant metabolic response to heat. Photosynthetic thermotolerance typically results from genotypic variation in several essential processes e.g., leaf net photosynthesis, transpiration stomatal conductance, and the thermostability of photosystems and electron transport. Other processes impacted include cell membrane thermostability (lipid remodelling), non-photochemical quenching protection, heat shock protein production, and the thermal liability of Rubisco activase. Heat typically modifies sucrose metabolism and increases rates of respiration. Whereas phloem sucrose export by sucrose exporters and/or import by sucrose transporters are inhibited in heat-stressed leaves, which further results in starch accumulation (Braun et al. 2014). The opposing rearrangements of starch metabolism with heat stress highlight the plasticity of starch metabolism, and reflects other factors e.g., tissue type developmental stage and growth (Thalman and Santelia 2017).

For the initial exploration of metabolic plasticity in the camelina panel, CVs were calculated for ten targeted leaf biomass compounds in response to heat and drought (Supplementary Table S5). CV is a measure of dispersion around the mean within a dataset which allows the exploration of phenotypic variability. In control conditions, the highest CV values (indicating a large dispersion among replicates and perhaps, plasticity within the study panel) were found for glucose, chlorophyll *b*, and starch, while the lowest CV values (low variability) were found for malate, polyphenols, and amino acids. Under abiotic stress, CV values of several major leaf compounds changed. CV of starch increased with drought and declined with heat; chlorophyll *b* and sucrose declined with both stresses; and glucose declined with heat. In contrast, CV values for polyphenols, amino acids, and chlorophyll *a* remained constant regardless of the treatment. The camelina panel thus displayed substantial variation in metabolic plasticity, which was modulated by abiotic stress.

Secondary metabolite multifunctionality can provide a better understanding of plant stress responses. The metabolomic analyses presented here identified the integral accumulation of plant secondary metabolites in the response of camelina to challenging environments. Metabolomic profiling showed that central metabolism of camelina responded differentially to the type of stress, with a more substantial effect of drought than heat. Untargeted analysis further confirmed the differential response to the different types of stress and showed that stress-responsive secondary metabolism distinguishes drought and heat treatments better than major compounds of plant biomass. In line with our results, seed metabolomic characterisation of six field-grown camelina genotypes showed high levels of plasticity depending on year and genotype (Boutet et al. 2022). Furthermore, high levels of plasticity in primary metabolites, including some sugars and major storage compounds such as fatty acids, proteins, and most lipid classes were found in two genotypes (Boutet et al. 2022). Classically stress compounds such as proline, γ-amino butyrate, polyamines, and branched-chain amino acid metabolism underpin the response of camelina to stress conditions. The biosynthesis of polyphenols (e.g., phenolic acids, flavonoids, stilbenoids and lignans) by camelina might alleviate the impact of stress-induced oxidative stress and the overproduction of ROS associated with environmental perturbation. Excess levels of ROS are a significant challenge, and camelina has multiple strategies to mitigate their impact. For example, estimates have indicated that camelina seeds have oxidative pentose phosphate pathway fluxes that exceed the demand for NAD(P)H for biosynthesis and are larger than those measured in other systems (Carey et al. 2020). It is possible that high levels of NAD(P)H in camelina could contribute to its antioxidative capacity.

Plant cells further respond to stress by undertaking remodelling of cellular lipid species to maintain cell integrity and functionality, exchanging FA (e.g., C18:1, C18:2 and C18:3) in membranes to maintain fluidity (Upchurch 2008). Camelina responded to heat and drought stress by significantly changing leaf FA composition; oleic acid showed a striking line-specific increase in response to heat, and decrease in drought, whilst linolenic acid declined with heat, indicating reduced membrane desaturation. These responses, mediated by FA desaturases (e.g., FAD2, FAD3) that are directly regulated by temperature, are typical of the cells' strategies to cope with abiotic stress (Gishini et al. 2025). However, the response of lipids to drought is less well characterized. FA are often remodelled in response to drought with levels of C18:3, C18:2 and C18:1 responding to maintain membrane fluidity. The decline in membrane fluidity is a disadvantage to crop growth under drought stress, because membrane fluidity plays an important role in stabilizing cellular metabolism and function. This study identified C18:1, along with C18:3 in seeds, as diagnostic markers for stress. This is consistent with recent camelina QTL mapping results in which markers associated with C18:1 were persistently detected in a diversity panel grown in multiple environmentally diverse locations. Furthermore, variation in C18:3 was associated with environmental temperature at the locations (Decker et al. 2025).The temperature response of monounsaturated fatty acids (C18:1), is often species and tissue specific, reflecting the regulatory control of Stearoyl-Acyl carrier protein Desaturase (SAD), which catalyses the first desaturation step leading to C18:1. Responses of C18:1 to stress e.g., heat and drought, likely reflect the combined regulatory control of Acyl-ACP thioesterase genes FATB and FATA (Byfield and Upchurch 2007). Remodeling fatty acids in molecular lipids is also achieved via the activity of lipases e.g., monoacylglycerol lipase (MAGL), involved in triacylglycerol breakdown. Expression of MAGL gene candidates has been correlated with the response to abiotic stress and oil assembly pathways (Kumar et al. 2025). Other lipase candidates responsive to heat include HEAT INDUCIBLE LIPASE1 (HIL1) which digests Monogalactosyldiacylglycerol (MGDG) to produce 18:3-free fatty acid. Like many crops (Higashi and Sato 2024), camelina has multiple remodelling activities to re-orientate the cellular lipidome and maintain cellular functionality and integrity. Lipid remodelling is a complex process of interacting activities, and a greater understanding of climate-related lipid remodelling will guide the development of lipid markers for resilient germplasm selection.

The efficiency with which camelina can reconfigure metabolic networks, resume active growth and establish a stress-tolerant state varied across the panel. The application of correlative approaches within metabolomics enabled the identification of metabolites (biomarkers) associated with stress-responsive states. Biomarkers of this type are often referred to as metabolic markers when derived from metabolite concentrations and have been recognised and used for the selection of optimised germplasm (Lamari et al. 2018; Xu et al. 2024). Camelina lines within the panel displayed a variety of response mechanisms, which included physiological adaptation and integrated cellular or molecular responses. For example, lines displaying a large range of metabolic strategies: some lines (e.g., UNT33, 32) had a significant metabolic stress response, whilst the lines with specific metabolic responses to drought and heat (e.g., UNT16, 5, 44, 45, 10) had only small amounts of adjustment. Drought was clearly associated with the broadest range of accumulated/depleted metabolic markers (1400 for UNT33 and zero for UNT26, 46, 56, 45, 7 and 44), which was nonetheless not correlated with field performance. Combinatorial assessment of the impact of stress on juvenile plants could identify responsive lines with large metabolic plasticity and good field agronomic performance e.g., UNT28, 18, but also lines categorised as unresponsive (e.g., UNT8) that also performed well in field environments. The moderate correlation observed between metabolomic profiles under drought stress (but not heat) and genomic structure (0.52, Fig. 6 B) suggested that genetic background only partly explained the metabolic response to drought. This indicated that some pathways or metabolites involved in drought tolerance were under genetic control and shared among related genotypes. This might also indicate that camelina is particularly well adapted to drought rather than heat, as indicated by the performance of the diversity panel in field trial located in France. The lower correlation between genomic and agronomic clustering (0.23) highlighted the fact that agronomic traits were probably influenced by more complex factors, such as plasticity or G x E interactions, which were not fully captured by genomic variation alone. Similarly, relatively low correlations between metabolomic profiles under different stress conditions (drought vs heat) showed that metabolic responses were quite specific to each environment, with different pathways activated depending on the stress, once again showing the plasticity of camelina to stress. The metabolic signatures thus provided a method to characterise this variation and identify specific camelina lines (Fernandez et al. 2016; Stasnik et al. 2024), thereby underscoring the crucial role of metabolomic studies in advancing research on orphan crops such as camelina. Despite its perceived lack of genetic diversity, camelina was nonetheless able to display remarkable plasticity in response to stress, providing an interesting leverage in crop improvement programs to identify improved ideotypes for specific pedoclimatic conditions.

Over recent years, research efforts have been dedicated to improving seed yield and agronomic traits in camelina (Blume et al. 2022; Blume et al. 2023). One approach was to tap into previously unexploited germplasm and characterise the population for traits e.g., juvenile abiotic stress tolerance, that will future-proof camelina for extreme climates, as shown in this study. After first examining and demonstrating the line-specific stress response in the diversity panel, we assessed how the measured diversity translated into agronomic performance in replicated multi-location field trials with different environmental challenges. Trait analysis of the diversity panel grown across the three trial locations demonstrated that the population contained lines capable of performing in different climates. The study confirmed the need to identify abiotic stress plasticity in camelina in combination with field testing. To capture both these approaches, scaled performance parameters were established e.g., TGW scaled by T_{min} (°C, at 50% flowering) linking environment measures that reflect heat or drought stress and crop traits. This approach could be deployed within a breeding strategy to develop climate resilient location-specific varieties. Furthermore, it could also be adopted for other ancestral crops as they become increasingly adopted by growers seeking to improve the climate resilience and diversity of their cropping systems.

Materials and Methods

Plant material

The 54 camelina lines were assembled from public collections (Supplementary Table S1). The panel was selected based on specific distinguishing features, namely genetic diversity, growth cycle length, performance under different climatic conditions, yield, and lipid profile. Prior to glasshouse experimentation and distribution for field trials, the panel was propagated in field trials of replicated plots at INRAe Versailles, France (Spring/Summer 2020).

Diversity panel population structure analysis

Leaf DNA was extracted using the Nucleospin Kit (Machery-Nagel, Düren, Germany) according to manufacturer. DNA was sequenced using paired end short read sequencing and analysed alongside whole genome re-sequencing data of publicly available camelina accessions (Luo et al. 2019, Li et al. 2021). The resulting data were trimmed with trimmomatic (Bolger et al. 2014) and mapped to camelina reference genome (PRJA264159). Alignment of sequencing reads was done with Burrows-Wheeler Aligner software (version 0.7.17). Genomic variants were identified through the characterisation of single nucleotide polymorphisms (SNPs) (GATK software tool version 0.7.44.1.4.0; Poplin et al. 2018). PLINK (Purcell et al. 2007) was used to carry out the principal component analysis (PCA) of camelina genetic diversity. ADMIXTURE was used to infer each accession's genome's fractional composition (Alexander et al. 2009), represented by bar plots where each accession was represented by a single bar sub-divided into coloured sections. Within ADMIXTURE a cross-validation assay to infer the error bias imposed by assuming the respective number of ancestral populations was performed. By comparison of this cross-validation error with runs using different numbers of ancestral populations the best fitting number of populations is then found as the one producing the lowest cross-validation-error (CVE). Eight populations were found to be optimal with an CVE of 0.47. Each section represents the fraction of a genome coming from ancestry belonging to the subpopulation represented by that colour. All code and plots used to carry out these analyses and a detailed description of the procedures are hosted on GitHub (https://github.com/usadellab/untwist). These files are also stored in the supplementary archive "Untwist_Population_Structure_Analysis.zip".

Trait measurements in early-stage drought and heat experiments

Three treatments were tested in the glasshouse: ambient temperature (20/18°C day/night) and well-watered (50% soil water content, SWC); ambient temperature (20/18°C day/night) and drought; and high temperature (32/25°C day/night) under well-watered conditions. SWC (each individual pot weighed) and BBCH scale (Biologische Bundesantalt, Bundessortenamt and Chemische Industrie; Martinelli and Galasso 2011) were monitored every day in all pots. Leaf length/width (cm) and leaf chlorophyll index (CI) measured using a portable self-calibrating chlorophyll meter SPAD (Chlorophyll Meter SPAD-502 Plus®, Konica Minolta Optics, Inc.), monitored every other day in all pots. Ambient and heat stress pots were kept at approx. 50% SWC. Drought was imposed by water withdrawal until each pot reached 15-20% SWC which maintained thereafter (Supplementary Fig. S1 A). Main stem width was measured with a calliper at the base of the shoot, and BBCH scale was derived from leaf number. BBCH and leaf length were used to monitor growth and stress impact, as it was considered that morphological changes (e.g., slower growth rate) were an indicator of stress. Leaf samples collected for analysis as detailed in Supplementary Methods.

Metabolomic analysis

Targeted and untargeted metabolomic techniques were used to cover primary and secondary leaf metabolites (Luna et al. 2020; Dussarrat et al. 2022). Robotised high-throughput ethanol extraction was used to obtain semi-polar metabolites from 20 mg of freshly frozen ground leaves (Luna et al. 2020). Quantitative profiling of major compounds of central metabolism included starch, sucrose, glucose, total proteins, total amino acids, malate, citrate, total polyphenols and chlorophylls *a* and *b*, using targeted assays (Poucet et al. 2021). Methods for untargeted metabolic profiling and analysis are detailed in the Supplementary Methods.

Total antioxidant capacity (TAC)

TAC was colourimetrically determined in a 96-well microplate format by using a commercial kit (Abbexa Ltd., Cambridge, UK; (Stasnik et al. 2022). Results were normalised as units (U) per mg protein in leaf extracts. Quadruplicate aliquots were used to quantify protein content using commercial ROTI®Quant Bradford solution (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) according to the supplier's instructions.

Fatty acid analysis

Field grown seed fatty acid (FA) analysis was performed at INRAe AgroParisTech, France, and at Rothamsted Research (RRes), UK; and leaf FA analysis of the glasshouse trials was performed at RRes. Both labs used FA methylation protocols analysed by gas chromatography detailed in Supplementary Methods. A reference camelina line was analysed by both labs, from which a linear equation was derived using the quantified amounts (µg/mg) of each FA. The linear equation was applied as a correction factor to the datasets, from which FA total content and relative composition were calculated.

Stable isotope determination

Carbon (C) and Nitrogen (N) content and their isotopic ratios were measured by an elemental analyser (EA, Flash 2000 Thermo Fisher Scientific) coupled with an isotopic ratio mass spectrometer (IRMS, Delta V Advantage Thermo Fisher Scientific), additional details are provided in the Supplementary Methods.

Camelina diversity panel multi-location field trials

Field trials were conducted in three different locations across Europe: Bologna (Italy), Versailles (France), and Harpenden (United Kingdom, UK). In Italy the diversity panel was sown on 29/11/2020 using a precision plot-drill at a rate of 500 seeds/m^2. The experimental layout was a completely randomized block with four replicates. In France, the diversity panel was sown on 26/03/2021 with a Wintersteiger Rowseed 1R single row seeder at a rate of 500 seeds/m^2. In both locations the experimental layout was a completely randomized block with four replicates. In UK, the diversity panel was sown on 19/04/2021 by shallow drilling the camelina seeds with a Haldrup S-25 drill, which followed a non-resolvable block design with four blocks of 56 plots each allowing for a total of up to five plot replicates per line. Further details, and a summary of the main dates and meteorological data by location can be found in Supplementary Methods and Table S7. Harvest was done at full maturity, all yield values were reported on a dry matter (DM) basis. Sub-samples of seed from each plot were collected and cleaned for further analysis.

Crop cycle and meteorological data

Location, sowing date, harvest date and meteorological data of the trials can be found in Supplementary Materials and Methods. Temperature (T) and precipitation data were collected by a weather station located onsite. Growing Degree Days (GDD) were calculated as $GDD=\Sigma[(T_{max}-T_{min})/2 - T_{base}]$, base temperature used was 4°C (Gesch and Cermak 2011).

Seed oil content

Seed oil content was measured by low-resolution time domain NMR spectroscopy using a Minispec MQ20 (Bruker) fitted with a robotic sample-handling system (Rohasys). The oil and moisture calibration were constructed according to the manufacturer's instructions using nine approximately 0.5 g oilseed seed samples ranging between 5% and 10% moisture content and between 30% and 55% oil content ($r^2 > 0.99$). Camelina seed with known oil and moisture content were supplied by Camelina Company España (CCE, Spain) for method verification. Approximately 1g of seed was used, seeds were kept in the room alongside the instrument for 24 hours prior to analysis to ensure equilibration with room temperature and humidity.

Thousand grain weight analysis

In Italy, TGW was determined using the Seed Counter S-25 machine by Data Technologies (DATA Detection Technologies Ltd., IL) at the Seed Research and Testing Laboratory (LaRAS) of the University of Bologna. In UK, TGW was determined using the MARVIN Digital Seed Analyser (MARViTECH GmbHGermany) to count the number of seeds in ~ 1g seed sample. In France, TGW was determined using the elmor C3 High Sensitive Seed Counter (Elmor, Switzerland).

Statistical analysis

The early-stage drought and heat stress experiment layout was a resolvable block design spreading the 54 panel lines over six blocks (trays); nine pots per tray. This was replicated five times and three independent randomisations done, one for each stress. Linear mixed models were fitted using REML to each variable with random structure accounting for the blocking imposed in the design (Compartment/Tray/Pot) and treatment structure Line * Stress. Approximate F-tests were calculated using the Satterthwaite approximation. All models were fitted in R using the lme4 and lmerTest packages. PCA of the effect of stress on measured traits, biomass components and parameters from the camelina diversity panel glasshouse early-stage study were implemented in R v.4.1 (R Core Team 2018) using BioStatFlow tools (v2.9). Multivariate analyses (PCA, Volcano plots) of metabolomic data were performed using MetaboAnalyst v5.0 (Pang et al. 2021) with normalised data (median normalisation, cube root transformation and Pareto scaling).

Accession Numbers

Further information related to the enzymes mentioned in this manuscript can be found in https://fatplants.net/home_.

Data availability

The data relating to the experiments described in this manuscript can be found in the Camelina Plant Adaptation Hub (https://www.camelina-hub.org/). Metabolomics raw data is available at https://www.ebi.ac.uk/metabolights/MTBLS9839, and the processed data can be found in https://doi.org/10.57745/ZAFPL9.

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Author contributions

RPH, YY, FZ, AM, BU, YG, JDF, CJ secured funding, conceived and designed the study. JP, PL, JDF provided the seeds for all trials. SS, EA, RPH performed all measurements, sampling, and data acquisition for the early-stage stress experiment. Field trials, sampling and data acquisition were completed by SS, EA, RPH in the UK; by BA, FZ in Italy; and by ADC, JDF in France. Lipid analysis was completed by SS, FT, EA, RPH, JDF. Sequencing and genotyping were performed by JDF, AH, BU; metabolomic analysis was completed by CC, PP, MLB, YG; stable isotope analysis by BA, FZ. TAC measured by DKG, CJ. Data handling and statistical analysis was completed by KH, SP, PP, AH, BU. SS, RPH produced the draft manuscript with edits from all authors.

Conflict of Interest

There was no conflict of interest with this work.

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Figure Legends

Figure 1. Genetic diversity in the study panel and publicly available camelina lines. (A) Scatterplot of the principal component analysis of publicly available (blue) and the study panel (red) camelina lines. Lines were abbreviated with a "C_" (public) or a "U_" (study panel) prefix followed by their accession number. (B) Admixture population genetic analysis (bottom) aligned with the hierarchical clustering dendrogram (top). Dendrogram generated from Identity-By-State distances; public camelina lines represented by their accession identifier (blue dendrogram leaves), study panel accessions with the "UNT" suffix (red dendrogram leaves). Admixture population genetics presented as a bar plot visualizing inferred genomic admixture for each camelina accession. Eight ancestral sub-populations were represented by eight different colors, each colored section in the bars represented the proportion of the accession's genome being inherited from the ancestral population indicated by color.

Figure 2. Metabolic changes in the camelina study panel in response to early-stage drought and heat stress. (A) Principal Component analysis (PCA) of ten major compounds using normalized targeted data (median normalization, cube root transformation and Pareto scaling). (B) Heatmap clustering analysis (Pearson's correlation, Ward clustering). All markers presented statistically significant variations (ANOVA, $P < 0.01$ with adjusted FDR correction). Each column represents the conditions (ambient, drought, heat) averaged for all samples. Each row indicates the major compounds with relative intensity shown as a heatmap (blue, depleted; red, accumulated). (C) PCA of normalized untargeted data (median normalization, cube root transformation and Pareto scaling) showing PC1 vs PC2 vs PC3. (A, C) Ambient, green triangle (Δ); Drought, blue plus sign (+); Heat, red circle (O). (D-E) Venn diagrams show specific and common metabolic markers identified by volcano plots ($FC > 2$; *t*-test $P < 0.01$, FDR corrected) that accumulated (D) or decreased (E) in response to drought or heat.

Figure 3. Response of metabolic markers to early-stage abiotic stress. (A) Drought, and (B) heat stress markers for each line in the camelina diversity panel plotted in the same order and color coded as determined by hierarchical clustering and admixture population genetics analysis shown in Fig. 1. Metabolic variables were qualified as markers if they were significantly accumulated or depleted when compared to ambient condition for a given camelina line (t-test, p-value < 0.05 FDR corrected; $\log_2 FC > 1$ or $\log_2 FC < 1$ respectively).

Figure 4. Leaf and seed fatty acid responses of the camelina diversity panel to early-stage environment ambient (red), drought (green), and heat (blue) treatments and field trials in diverse pedoclimates (France - yellow, heat; Italy - purple, drought & UK - orange, temperate).

Relationship between total fatty acids and relative polyunsaturated fatty acids (PUFA) ratio for individual lines/replicates leaves for each treatment (A) and field grown seeds (B). Leaf C18:1 (C) and C18:3 (D – leaf; E- field seed) response to treatment for individual lines (x axis) plotted and color coded based on the distributions determined hierarchical clustering and admixture population genetics analysis shown in Fig. 1. Data was transformed to ensure assumptions of homogeneous variance hold and in the case of D) original scale shown on the right-hand side. Error bars illustrate least significant difference (LSD) between lines (solid black) and between stresses (dashed black). Interaction between stress and line was non-significant for leaf C18:3 (D). (E) line mean ±standard error shown, ITA and FR N=4, UK N≤15.

Figure 5. Principal component analysis (PCA) on measured traits from the camelina diversity panel early-stage abiotic stress study. Drought over ambient (A, B) and heat over ambient (C, D) for the individual lines (A and C, colors represent cos2 values, a proxy for the quality of the representation in the 2D space, ratios were scale-normalized before performing the PCA, blue shading highlights 'unresponsive' lines) and the measured variables (B and D, colors represent the contribution of the variables to the PCA: leaf fatty acids, chlorophyll content (SPAD), stable isotope (Δ^{15}N, Δ^{13}C), phenology (BBCH scale, stem thickness, leaf number, width, length), soil water content (SWC), fresh and dry weight, plant water content and major biomass components: glucose, sucrose, starch, malate, citrate, total polyphenol content, free amino-acid, total protein content, chlorophyl *a* and *b*).

Figure 6. (A) Relationship between metabolomic clustering and agronomic performance under different environmental conditions. For each of the three glasshouse conditions tested (control, drought and heat stress), genotypes (color coded after Fig 1.) were clustered based on their metabolomic profiles using hierarchical clustering. Agronomic parameters (seed yield (g/m^2), thousand grain weight (TGW, g), and seed oil content (% of seed weight) were represented as color gradients aligned with the dendrogram tips. (B) Comparison of dendrogram similarity between phylogenetic, agronomic and metabolomic data. Correlation plot showing pairwise Baker's Gamma correlation coefficients between dendrograms derived from phylogenetic, agronomic, and metabolomic data (from early-stage experiments: control, drought and heat stress). Each coefficient quantified the structural similarity between two dendrograms, with values ranging from –1 (complete discordance) to 1 (perfect agreement). Numerical values of the coefficients are shown in the lower triangle. The upper triangle displays a graphical representation of the correlation strength.

Figure 7. Summary of the multilevel stress responses *Camelina sativa* deploys to abiotic stress (heat and drought). Selected variables are shown to indicate the overall

morphophysiological (phenology, physiology and metabolic) response shown by juvenile leaves to treatment, as indicated in earlier figures (Fig 2-5, Supplementary Fig. S2, S4). These responses may vary between lines. Red arrow, increase; blue arrow, decrease; grey dot, non-significant change. BBCH, scale of phenological growth stages; Δ^{13}C, and Δ^{15}N leaf stable isotope analysis; TAC, total antioxidant capacity; PUFA, polyunsaturated fatty acids. Scale bar = 5 cm.

Tables

Table 1. Summary of the metabolic response of the camelina diversity panel to early-stage abiotic stress (drought and heat) measured in juvenile leaves. Stress responsive markers (common and stress specific) for individual metabolic pathways are shown, alongside the total annotated. Orange- and blue-colored numbers show highest increased and depleted markers respectively.

	Common markers		**Drought-specific markers**		**Heat-specific markers**	
Metabolic pathways	Increased	Depleted	Increased	Depleted	Increased	Depleted
Alcohols and polyols	1	5	2	2	0	3
Alkaloids	0	2	0	0	4	2
Amino acids and derivatives	3	7	16	6	4	3
Benzenoids	**17**	5	5	**17**	13	12
Carbohydrates and derivatives	**28**	3	14	9	12	9
Hydrocarbon derivatives	1	2	0	2	0	0
Indoles	2	0	0	0	0	0
Lignans	1	2	3	0	1	12
Lipids and derivatives	3	7	11	11	8	3
Nucleotides and derivatives	2	3	2	11	2	2
Organic acids and derivatives	4	16	0	**17**	2	**17**
Organoheterocyclic compounds	5	10	14	6	4	8
Phenylpropanoids and polyketides	**26**	**36**	**34**	**17**	**35**	**28**
Terpenes	6	2	0	4	15	2
Total annotated	**94**	**58**	**64**	**54**	**85**	**65**

Table 2. Seed fatty acid composition for the camelina grown in multi-location field trials. Fatty acids as number of carbons:desaturations (% of total FAMES); FR, France (N=216); ITA, Italy (N=216); UK, United Kingdom (N=672); results show average for all lines, ± standard error).

Trial	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:1
FR	6.46 ±0.020	2.59 ±0.012	15.26 ±0.095	18.08 ±0.077	31.75 ±0.092	1.72 ±0.010	15.85 ±0.041	2.01 ±0.016	1.36 ±0.011	3.23 ±0.025
ITA	6.02 ±0.019	2.65 ±0.012	15.45 ±0.077	17.23 ±0.076	32.97 ±0.074	1.62 ±0.011	15.94 ±0.044	1.91 ±0.014	1.40 ±0.008	3.16 ±0.024
UK	5.91 ±0.042	2.53 ±0.012	15.03 ±0.104	18.59 ±0.091	34.70 ±0.098	1.35 ±0.008	14.12 ±0.061	1.91 ±0.012	1.39 ±0.009	2.62 ±0.020

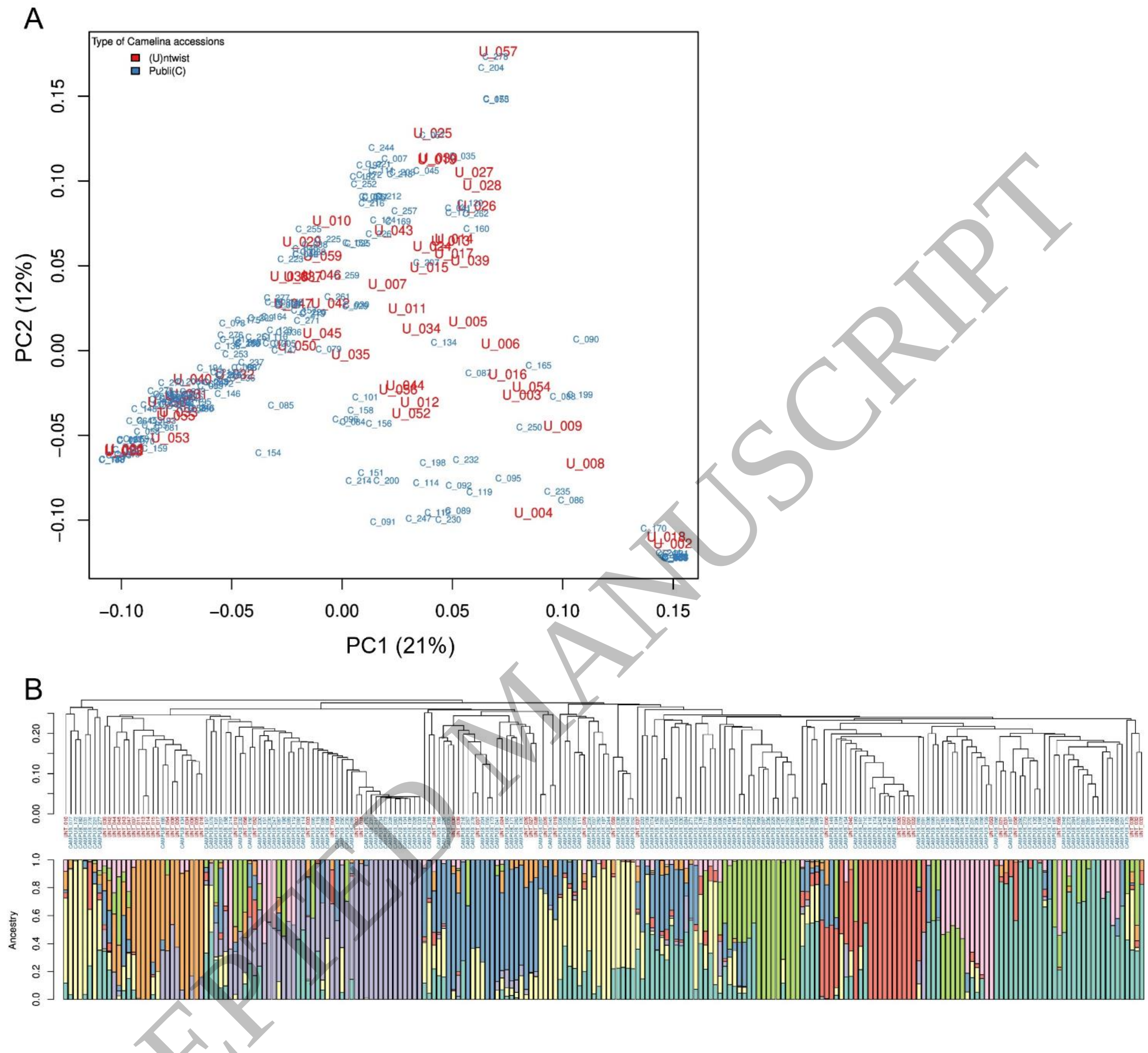
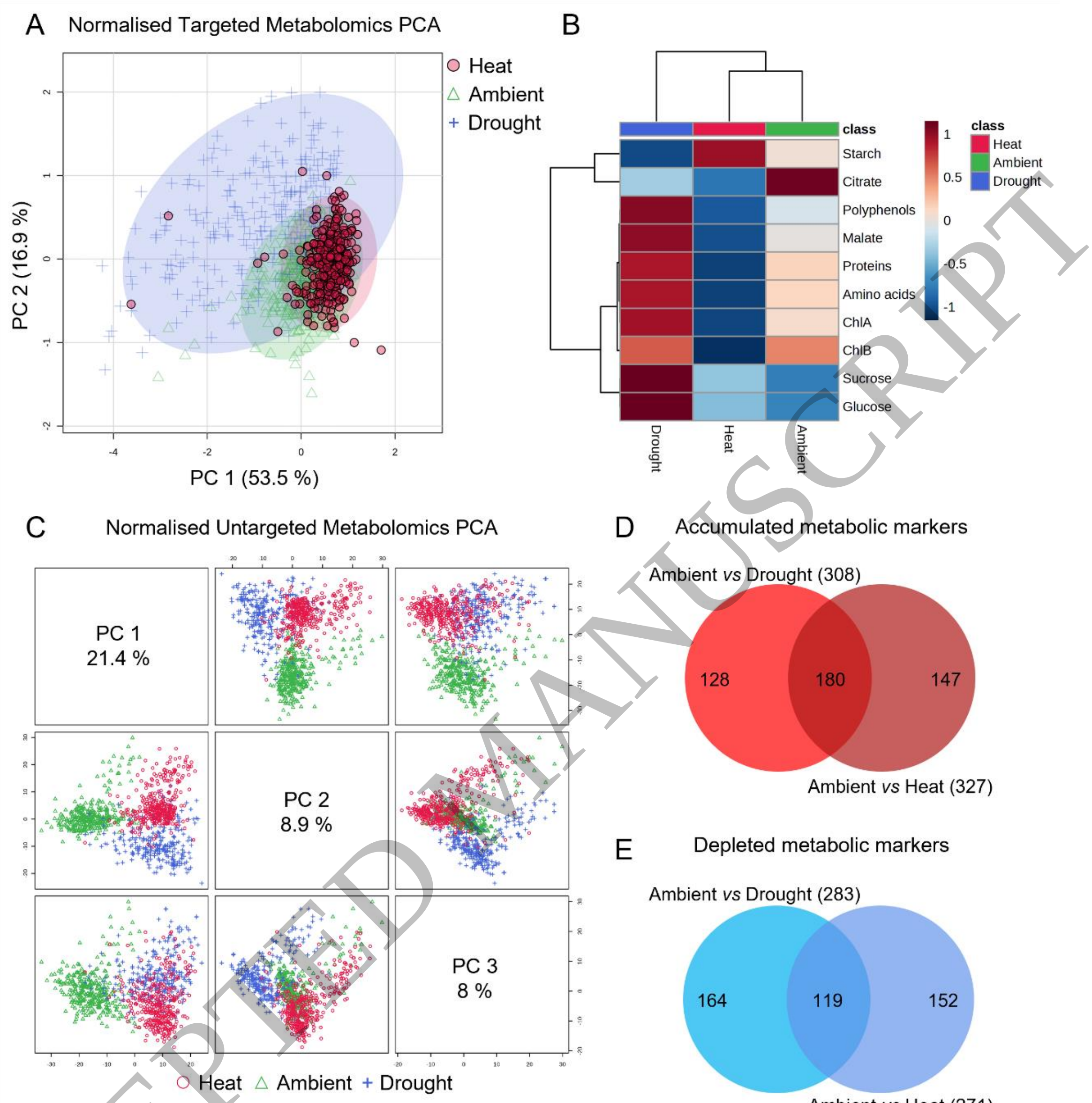


Figure 1
175x159 mm (x DPI)

1



2 *Figure 2*

3 *150x159 mm (x DPI)*

4

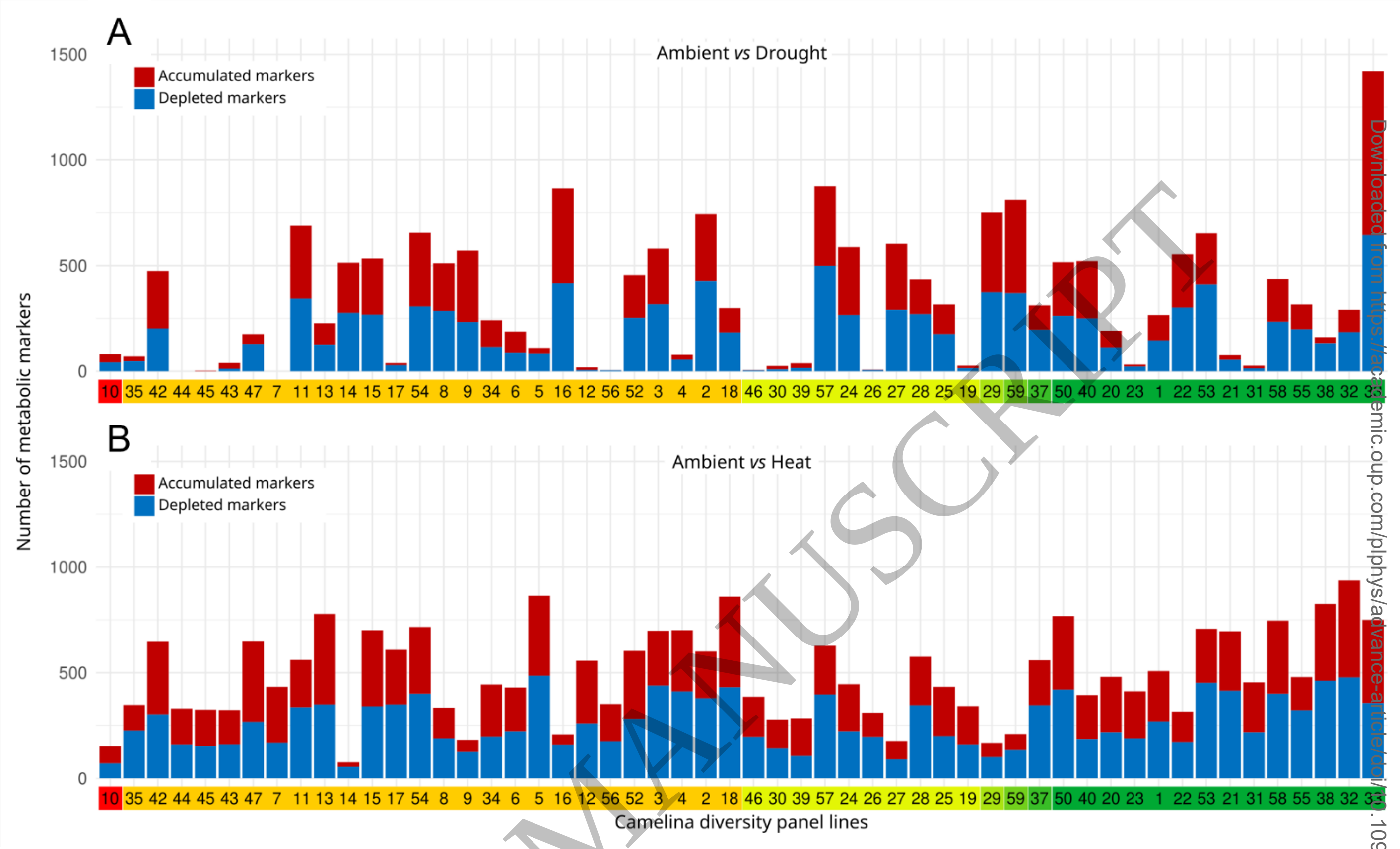


Figure 3
187x113 mm (x DPI)

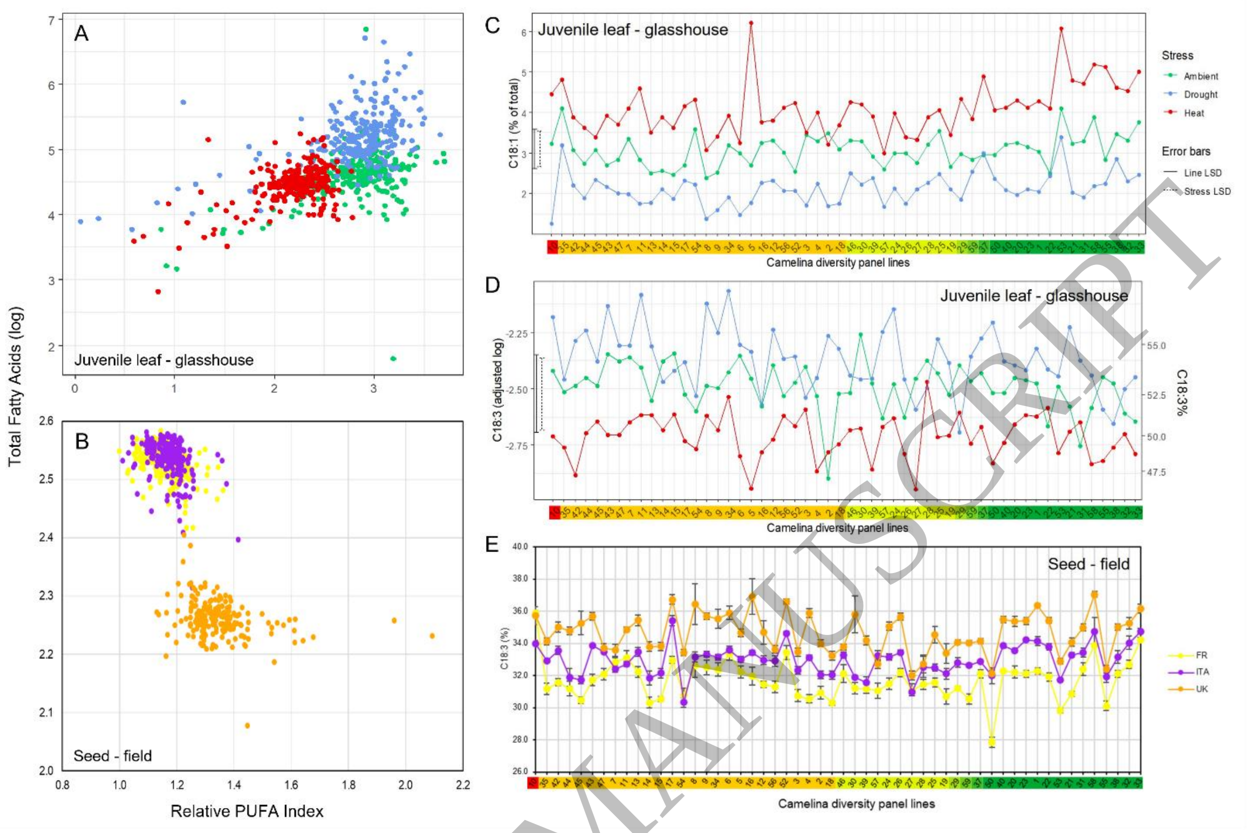


Figure 4

238x159 mm (x DPI)

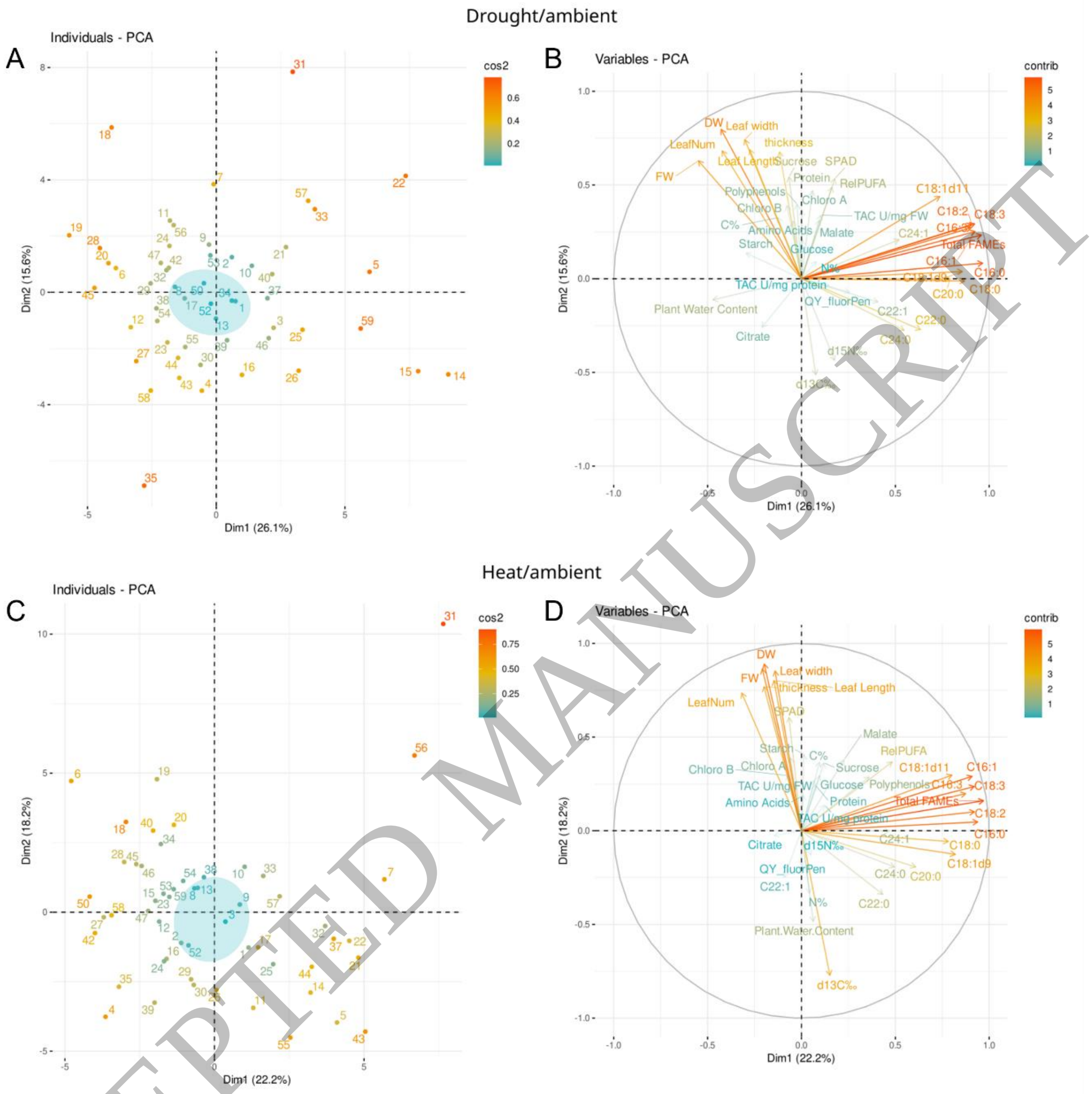


Figure 5
157x159 mm (x DPI)

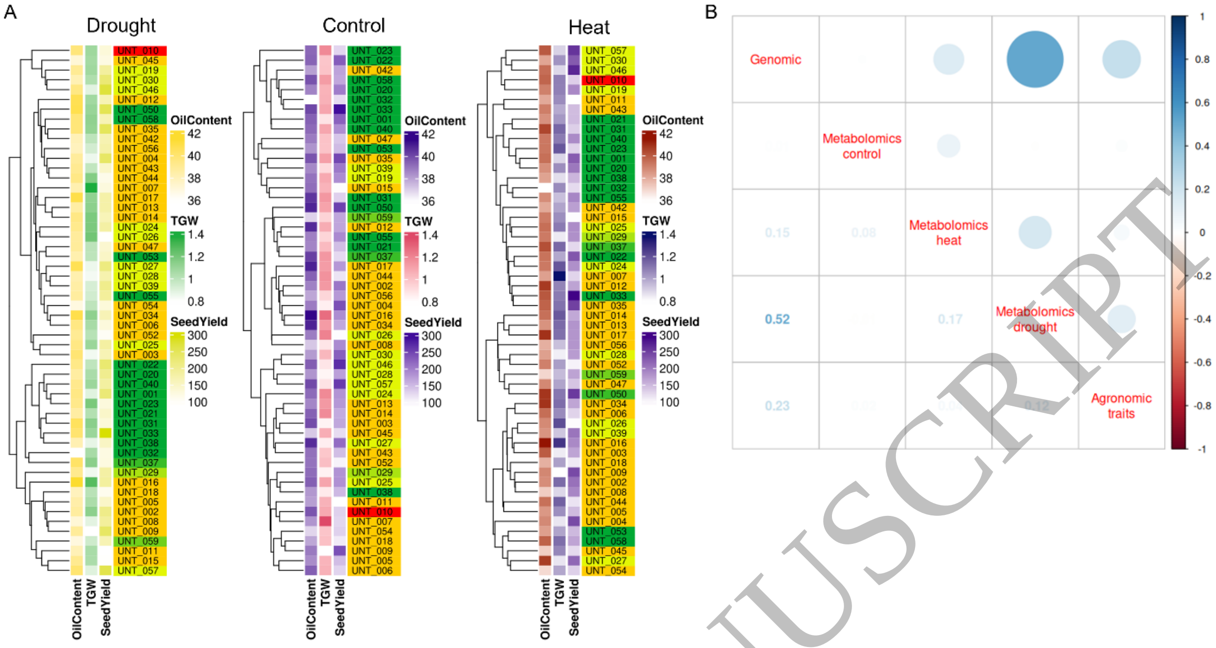
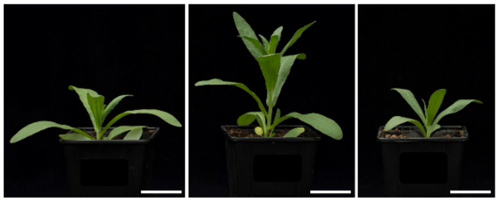


Figure 6

246x132 mm (x DPI)



Phenology	Control	Heat	Drought
BBCH		↑	↓
Leaf length		●	↓
Leaf width		●	↓
Physiology			
$\Delta^{13}C$		●	↑
$\Delta^{15}N$		↑	●
Plant water content		●	↓
TAC		↑	●
Metabolites			
Polyphenols		↓	↑
Malate		↓	↑
Proteins & Amino acids		↓	↑
Chlorophyll		↓	↑
Simple sugars (sucrose & glucose)		↓	↑
Citrate		↓	↓
Starch		↑	↓
Oleic acid		↑	↓
Linolenic acid		↓	↑
Total fatty acids		●	↑
Relative PUFA content		↓	●

Figure 7
94x159 mm (x DPI)