

Article

Comparative Analysis of the Growth, Physiological Responses, and Phenolic Compounds of *Thymus* Species Under Drought Stress in Field and Greenhouse Conditions

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Abstract

Drought stress can substantially affect growth and secondary metabolite production in medicinal plants. This study evaluated the growth, physiological responses, and phenolic compound contents of ten *Thymus* populations under greenhouse and field conditions with three irrigation levels: control, moderate stress, and severe stress (corresponding to 100%, 70% and 40%, respectively, of field capacity). Drought significantly reduced plant height, biomass, and relative water content, with *T. serpyllum* and *T. daenensis-1* showing greater reductions in several growth and physiological traits. In contrast, *T. kotschyanus-2* and *T. vulgaris* generally maintained relatively better growth and water status. Under stress, proline accumulation and electrolyte leakage increased, while photosynthetic pigments declined and carotenoid levels increased in several populations. Total phenolic and flavonoid contents generally increased under drought, particularly in *T. vulgaris* and *T. kotschyanus-2*. Rosmarinic acid, the predominant phenolic compound detected, increased significantly under drought stress. Essential oil content ranged from 0.33% to 2.50% across populations and environments and showed population-dependent responses to drought, with increases observed in several populations. Multivariate analyses and drought tolerance indices identified *T. kotschyanus-2* as showing relatively better drought performance among the evaluated populations, based on its relatively smaller reductions in biomass, higher relative water content, and favorable biochemical responses. In contrast, *T. serpyllum* showed comparatively greater sensitivity to drought across several measured traits. Drought reduced growth while enhancing the accumulation of several phenolic and other specialized metabolites, suggesting a potential shift in resource allocation between growth and secondary metabolism. *T. kotschyanus-2* showed relatively better drought performance, suggesting its potential for further evaluation under water-limited cultivation conditions. Comparison of the greenhouse and field experiments revealed common drought-response patterns as well as environment-dependent differences in their magnitude.

Keywords: *Thymus* spp.; drought tolerance; secondary metabolites; polyphenols components; essential oil; multivariate analysis



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1. Introduction

Drought is one of the most critical abiotic stresses that limit plant growth, productivity, and survival [1,2]. Drought-tolerant plants may maintain growth and water status through coordinated physiological and biochemical responses, including osmotic adjustment and

the accumulation of compatible solutes [3]. Drought-stressed plants often experience reduced growth [4–6] and altered metabolite composition [7–9]. Tolerant genotypes may exhibit a lower accumulation of some stress-induced metabolites while enhancing the production of osmolytes that contribute to osmotic adjustment [3]. Therefore, increasing drought frequency and severity associated with climate change may significantly impact the cultivation and harvesting of medicinal plants [3,4,10,11].

Thymus is a herbaceous perennial or subshrub belonging to the Lamiaceae family. Thyme is an important aromatic and medicinal plant, consumed in different parts of the world and widely used in traditional medicine in the Mediterranean and the Middle East [12]. To date, 214 species and 36 subspecies are recognized [13]. The genus includes 18 species within the Iranian flora, four of which are endemic to Iran [14]. The aerial parts have been used in traditional medicine due to their carminative, anti-inflammatory, antioxidant, antibacterial, antifungal, and expectorant properties [15]. Thyme is particularly valued for its rich content of bioactive secondary metabolites, including essential oils (mainly thymol and carvacrol), phenolic acids such as rosmarinic acid, and various flavonoids (e.g., luteolin and apigenin derivatives) [16]. The biosynthesis and accumulation of these metabolites are strongly influenced by genetic and environmental conditions, particularly drought stress, which may significantly alter both their quantity and composition [16,17], and different thyme species respond differently to drought stress [18,19]. Similarly, comparative studies of Lamiaceae species have shown species-specific responses to drought in terms of biomass and essential oil content [20–22].

While some previous studies have reported molecular and polyphenolic variation among Iranian thyme populations [23], the morphological characteristics and oil content of the leaves of this genus have only been assessed in a specific geographical region in south-eastern Iran [15,24]. Despite the existing research on molecular and metabolic diversity among Iranian thyme populations, comprehensive evaluations integrating morphological, physiological, biochemical, and phytochemical responses under different drought regimes remain scarce. Furthermore, population-dependent variation in drought responses under controlled greenhouse and open-field environments remains poorly understood in *Thymus* spp.

Recent research underscores the importance of considering environmental variation when interpreting drought responses in medicinal and aromatic plants. A recent meta-analysis of the effects of drought on medicinal species revealed substantial variation in physiological and biochemical responses, including yield, chlorophyll content, antioxidant activity, and essential oil production, across studies and environmental conditions. This emphasizes that drought responses are not uniform and may depend on both population characteristics and environmental conditions [25]. Recent evaluations of diverse *Cymbopogon* genotypes revealed significant effects of genotype, environment, and their interaction on essential-oil yield and quality-related traits, with some genotypes showing greater stability across environments [26]. In *Origanum vulgare* subsp. *hirtum*, genotype $\times$ environment interactions significantly affected essential-oil yield across Mediterranean environments and years, demonstrating that environmental variation can substantially modify both productivity and genotype performance [27]. These findings highlight the value of evaluating *Thymus* populations under contrasting environmental conditions to identify populations showing relatively better drought performance.

Examining plant responses solely in greenhouses or fields may yield incomplete results. Greenhouse experiments allow for the precise control of environmental variables and facilitate the identification of intrinsic physiological and biochemical responses to drought. Field trials, on the other hand, reflect realistic agronomic conditions and environmental variability. Therefore, evaluating both environments provides complementary information

on population performance under controlled and field conditions and allows common and environment-dependent drought-response patterns to be examined descriptively.

Therefore, the present study investigated the morphological, physiological, biochemical, and phytochemical responses of ten thyme (*Thymus* spp.) populations under different levels of drought stress under field and greenhouse conditions. Integrating drought tolerance indices, multivariate analyses, and metabolite profiling enabled the identification of populations showing relatively better drought performance and distinct phytochemical responses. The findings provide a basis for further evaluation of drought-resilient *Thymus* populations under water-limited cultivation conditions.

2. Results

2.1. Morphological Responses of Ten *Thymus* Populations to Drought Under Field Conditions

First, we evaluated the morphological responses of ten thyme populations to three irrigation levels under field conditions. Analysis of variance (see Table S2) revealed that population and irrigation level significantly influenced all measured traits. Significant population $\times$ irrigation interactions for internode length, shoot dry weight, and leaf length ($p \leq 0.05$) suggest that the populations responded differently to increasing drought intensity.

Under well-watered conditions, several populations exhibited vigorous growth. *T. pubescens* produced the longest internodes and the highest shoot dry weight. However, progressive water limitation markedly reduced growth in most populations. Under MWS and SWS, internode length, leaf length, and biomass generally declined, reflecting restricted vegetative development.

The reduction in shoot dry weight varied among populations, with some populations showing smaller declines under water limitation than others. For example, *T. kotschyanus*-2 exhibited relatively smaller decreases in biomass and leaf size under stress than several other populations. In contrast, *T. daenensis*-1 and *T. serpyllum* showed relatively greater reductions in several growth traits under drought (Table 1).

Overall, the magnitude of reductions in biomass and leaf development varied among populations under reduced irrigation, indicating population-dependent variation in morphological responses to water limitation.

2.2. Physiological Responses of Ten *Thymus* Populations to Drought Under Field Conditions

To further characterize the physiological responses to water limitation, we assessed physiological traits under different irrigation regimes. Analysis of variance identified significant effects of population and irrigation on all measured variables, while significant population $\times$ irrigation interactions were observed for most variables ($p \leq 0.05$). This finding indicates population-specific variation in physiological responses to drought (see Table S3). However, irrigation had no significant effect on the chlorophyll a/b ratio.

Table 1. Mean comparisons of morphological traits of ten thyme populations after the application of different irrigation regimes under field conditions.

Populations	Plant Height (cm)			Internode Length (cm)			Shoot Fresh Weights (g)			Shoot Dry Weights (g)			Leaf Length (cm)			Leaf Width (cm)		
	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS
Tm	20 ^{a-f}	19.33 ^{a-g}	16 ^{d-g}	0.86 ^{b-e}	0.90 ^{b-e}	0.86 ^{b-e}	3.63 ^{a-f}	3.16 ^{c-i}	2.13 ^{k-n}	2.10 ^{a-d}	1.80 ^{c-g}	1.03 ^{h-m}	0.80 ^{h-j}	0.63 ^{ij}	1 ^{e-h}	0.50 ^a	0.33 ^{a-d}	0.33 ^{a-d}
Tf	18 ^{b-g}	17 ^{c-g}	18 ^{b-g}	0.76 ^{b-e}	0.63 ^{c-e}	0.73 ^{c-e}	3.50 ^{a-g}	3.20 ^{c-i}	2.43 ^{i-m}	1.60 ^{d-i}	1.16 ^{h-l}	0.90 ^{j-n}	0.56 ⁱ	0.50 ^j	0.50 ^j	0.43 ^{a-c}	0.40 ^{a-d}	0.33 ^{a-d}
Ts	26 ^a	24.33 ^{ab}	17 ^{c-g}	0.96 ^{b-d}	0.73 ^{c-e}	0.73 ^{c-e}	3.88 ^{a-d}	3.06 ^{c-j}	2.70 ^{g-l}	2.50 ^{ab}	1.36 ^{e-k}	0.76 ^{k-n}	1.46 ^{a-d}	1.43 ^{a-d}	1.46 ^{a-d}	0.23 ^{cd}	0.23 ^{cd}	0.20 ^d
Tp	23.66 ^{a-c}	20 ^{a-f}	19.33 ^{a-g}	1.50 ^a	0.76 ^{b-e}	1.06 ^{a-c}	3.46 ^{a-h}	2.83 ^{e-l}	2.23 ^{j-n}	2.56 ^a	1.53 ^{d-j}	0.76 ^{k-n}	1.50 ^{a-c}	1.36 ^{a-d}	1.36 ^{a-d}	0.40 ^{a-d}	0.30 ^{a-d}	0.30 ^{a-d}
Td1	22.66 ^{a-d}	19 ^{b-g}	19 ^{b-g}	1.26 ^{ab}	0.50 ^{de}	0.66 ^{c-e}	2.40 ^{i-m}	2.16 ^{j-n}	1.56 ^{mn}	0.93 ⁱ⁻ⁿ	0.63 ^{l-n}	0.33 ⁿ	1.66 ^a	1.30 ^{b-e}	1.30 ^{b-e}	0.40 ^{a-d}	0.30 ^{a-d}	0.23 ^{cd}
Td2	24.66 ^{ab}	24.33 ^{ab}	22.33 ^{a-e}	1 ^{a-d}	1 ^{a-d}	0.80 ^{b-e}	2.77 ^{f-l}	2.10 ^{ln}	1.46 ⁿ	1.29 ^{f-l}	0.93 ⁱ⁻ⁿ	0.43 ^{mn}	1.56 ^{ab}	1.43 ^{a-d}	1.23 ^{c-f}	0.36 ^{a-d}	0.40 ^{a-d}	0.23 ^{cd}
Tfed	16 ^{d-g}	14.33 ^{fg}	13 ^g	0.63 ^{c-e}	0.60 ^{c-e}	0.53 ^{de}	3.26 ^{b-i}	3.03 ^{d-k}	2.23 ^{j-n}	2.46 ^{a-c}	2.03 ^{a-e}	1.13 ^{h-l}	1.16 ^{d-g}	1.03 ^{e-g}	0.96 ^{f-h}	0.36 ^{a-d}	0.43 ^{a-c}	0.26 ^{b-d}
Tkot1	23.33 ^{a-c}	20.33 ^{a-f}	17.33 ^{c-g}	0.50 ^{de}	1 ^{a-d}	0.63 ^{c-e}	3.98 ^{a-c}	3.73 ^{a-e}	2.60 ^{g-l}	2.15 ^{a-d}	1.70 ^{d-h}	1.23 ^{f-l}	0.96 ^{f-h}	0.90 ^{hi}	1.26 ^{b-f}	0.46 ^{ab}	0.43 ^{a-c}	0.40 ^{a-d}
Tkot2	15.66 ^{e-g}	14 ^{fg}	13.66 ^{fg}	1.06 ^{a-c}	0.43 ^e	0.50 ^{de}	4.34 ^a	4.16 ^{ab}	3.30 ^{b-i}	2.43 ^{a-c}	2.20 ^{a-d}	1.30 ^{f-l}	1.03 ^{e-h}	1.20 ^{c-g}	1.20 ^{c-g}	0.50 ^a	0.40 ^{a-d}	0.40 ^{a-d}
Tv	22 ^{a-e}	21.33 ^{a-e}	20.33 ^{a-f}	0.63 ^{c-e}	0.76 ^{b-e}	0.56 ^{c-e}	2.54 ^{h-l}	2.23 ^{j-n}	1.66 ^{mn}	1.84 ^{b-e}	1.70 ^{d-h}	1.23 ^{f-l}	0.73 ^{h-j}	0.60 ^{ij}	0.60 ^{ij}	0.30 ^{a-d}	0.30 ^{a-d}	0.33 ^{a-d}

C, control; MWS, moderate water stress; SWS, severe water stress. Tm: *T. migricus*, Tf: *T. fallax*, Ts: *T. serpyllum*, Tp: *T. pubescens*, Td1: *T. daenensis-1*, Td2: *T. daenensis-2*, Tfed: *T. fedtschenkoi*, Tkot1: *T. kotschyanus-1*, Tkot2: *T. kotschyanus-2*, Tv: *T. vulgaris*. *n* = 3, the letters are based on the least significant difference (LSD) test at the 5% probability level.

Drought stress affected photosynthetic pigments differently among populations. In several populations, chlorophyll a, chlorophyll b, and total chlorophyll decreased under SWS. For instance, total chlorophyll decreased by 34% in *T. kotschyanus-2*, whereas carotenoid content decreased by 79% in *T. migricus* under SWS. In contrast, *T. fallax* and *T. daenensis-1* maintained or increased their pigment content by up to 74% and 61%, respectively, compared with the control. The chlorophyll a/b ratio varied among populations. Higher values were observed in *T. pubescens*, *T. daenensis-1*, and *T. daenensis-2*, while lower ratios were recorded in *T. migricus* and *T. kotschyanus-2*. However, irrigation level did not significantly affect the chlorophyll a/b ratio. The largest increases relative to the control were observed in *T. fallax* and *T. pubescens* under SWS (Table S4). All thyme populations showed increased proline concentrations in response to drought stress. *T. pubescens* exhibited the highest increase of 81% relative to the control, while *T. fallax* showed the lowest increase of 11% (Table S5). Drought stress caused a reduction in RWC in all populations examined. *T. daenensis-1* demonstrated the greatest decrease in RWC (51%) under SWS, whereas *T. migricus* and *T. fedtschenkoi* demonstrated the smallest decreases (Table S5). EL increased significantly under water stress in all thyme populations. The largest increases in EL were observed in *T. migricus* (80%) and *T. vulgaris* (100%), while the smallest increase was seen in *T. serpyllum* (6%) under SWS (Table S5).

Overall, the magnitude and direction of physiological responses to drought varied among populations. Populations differed in their ability to maintain tissue water status and photosynthetic pigments and in the extent of electrolyte leakage under water limitation, indicating population-dependent variation in physiological responses.

2.3. Biochemical Responses of Ten Thymus Populations to Drought Under Field Conditions

Next, we evaluated the effects of drought on biochemical and secondary metabolism-related traits. Analysis of variance revealed significant effects of population and irrigation, as well as their interaction, on all measured parameters ($p \leq 0.05$). This result indicates population-dependent variation in biochemical responses to drought stress (Table S6). Generally, increasing drought severity enhanced TFC, TPC, DPPH, and EO content; the magnitude and direction of these responses varied among populations. However, the magnitude of these increases varied markedly among populations. *T. kotschyanus-2* showed relatively large increases in several biochemical traits, including relatively large increases in several biochemical traits, including TFC and TPC (up to 71% for total phenols), and relatively high antioxidant activity, as indicated by lower IC₅₀ values. In contrast, *T. fedtschenkoi* and *T. daenensis-2* exhibited only modest increases in phenolics and flavonoids (approximately 11 to 25%) and comparatively higher IC₅₀ values (Table S7).

Essential oil content also varied among populations. EO content ranged from 0.59% to 2.50% under field conditions and varied among populations and irrigation regimes. EO content increased under drought conditions in several populations, particularly in *T. kotschyanus-2* and *T. fedtschenkoi*, which showed increases of 87% and 75%, respectively, under SWS. Conversely, *T. pubescens* displayed only a slight increase under MWS (Table S7).

Overall, the magnitude and direction of biochemical responses to drought varied among populations, with differences observed in phenolic and flavonoid accumulation, antioxidant activity, and EO content.

To further characterize multivariate patterns of physiological and biochemical variation among the ten thyme populations, principal component analysis (PCA) was conducted. The first two components accounted for 38.4% and 26.71% of the total variation under control conditions (Figure 1a). PC1 was positively associated with EL, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. This PC was named photosynthetic pigment

potential. PC2 was positively associated with EL, proline, and DPPH, indicating a distinct pattern of variation in stress-related and antioxidant-associated traits (Figure 1a). Under MWS conditions, a similar multivariate pattern was observed. PC1 was positively associated with EL, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. Proline and DPPH were highly associated with PC2 (Figure 1b).

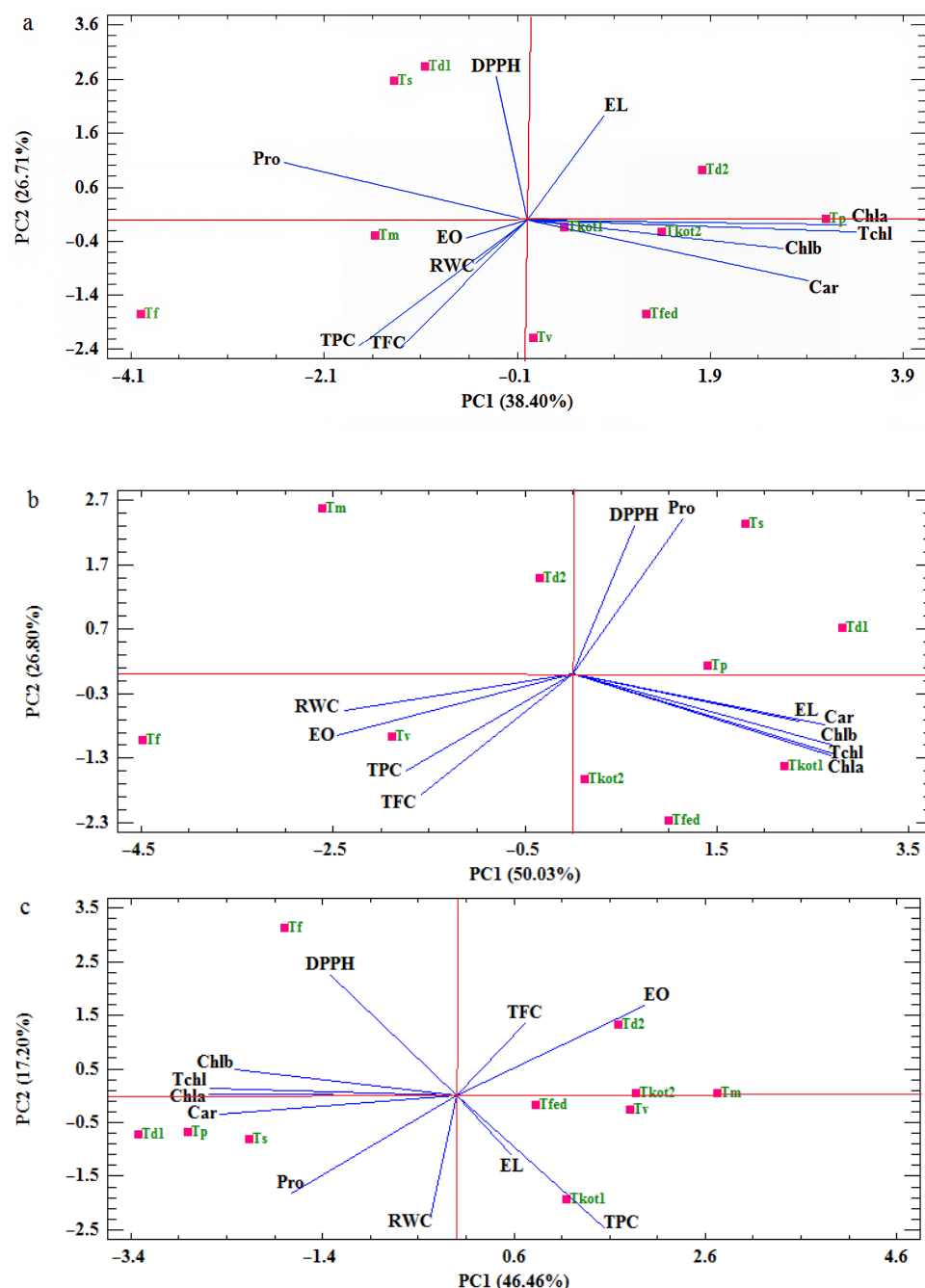


Figure 1. Principal component analysis based on biochemical and physiological traits of ten thyme populations, Tm: *T. migricus*, Tf: *T. fallax*, Ts: *T. serpyllum*, Tp: *T. pubescens*, Td1: *T. daenensis-1*, Td2: *T. daenensis-2*, Tfed: *T. fedtschenkoi*, Tkot1: *T. kotschyanus-1*, Tkot2: *T. kotschyanus-2*, and Tv: *T. vulgaris*, after the application of different irrigation regimes: (a) control, (b) moderate water stress, and (c) severe water stress under field conditions. TPC: total phenols, TFC: total flavonoids, EO: essential oil, EL: electrolyte leakage, RWC: relative water content, Pro: proline, Chla: chlorophyll a, Chlb: chlorophyll b, Tchl: total chlorophyll, and Car: carotenoids. Point labels: number of complete cases, vectors: data variables.

Under SWS, the first two components accounted for 46.46% and 17.2% of the total observed variation (Figure 1c). PC1 showed a strong correlation with EL, TFC, TPC, and EO content. PC2 was positively associated with chlorophyll a, chlorophyll b, total chlorophyll, DPPH, TFC, and EO content, reflecting variation in photosynthetic and biochemical traits among populations (Figure 1c).

In the PCA biplots, *T. fedtschenkoi*, *T. kotschyanus-1*, *T. kotschyanus-2*, and *T. daenensis-2* showed relatively similar multivariate patterns under both control and SWS conditions. Similarly, *T. serpyllum* and *T. daenensis-1*, as well as *T. vulgaris* and *T. migricus*, showed similar positions in the PCA space under both conditions. The other populations clustered separately (Figure 1).

2.4. Drought Tolerance Indices of Ten *Thymus* Populations

Analysis of variance revealed significant differences ($p \leq 0.05$) among thyme populations for shoot dry weight under non-stress (Y_p) and drought-stress (Y_s) conditions and for most drought tolerance indices, whereas SSI and YSI did not differ significantly among populations (Table S8). These results indicate substantial population-level variation in biomass production and drought-response characteristics. *T. vulgaris*, *T. pubescens*, *T. fedtschenkoi*, *T. kotschyanus-1*, and *T. kotschyanus-2* had relatively high shoot dry weight under non-stress conditions (Y_p) values, whereas *T. daenensis-1* had the lowest Y_p . The highest shoot dry weight under stress conditions (Y_s) values were observed in *T. pubescens*, *T. fedtschenkoi*, *T. kotschyanus-1*, *T. kotschyanus-2*, *T. serpyllum*, and *T. migricus*, while *T. daenensis-1* showed the lowest Y_s (Table S9). The STI values were highest in *T. kotschyanus-2*, *T. fedtschenkoi*, and *T. pubescens*, whereas *T. daenensis-1* had the lowest STI values (Table S9). SSI was highest in *T. serpyllum*, followed by *T. pubescens* and *T. daenensis-1*, while *T. vulgaris* had the lowest SSI value. YSI was highest in *T. vulgaris* and lowest in *T. serpyllum*. These indices provided complementary information on drought response, with *T. kotschyanus-2* showing relatively favorable values for several performance-related indices, whereas *T. serpyllum* showed a relatively high susceptibility profile based on its high SSI and low YSI values (Table S9).

Correlation analysis further demonstrated significant positive associations between Y_p and Y_s ($r = 0.74^{**}$), indicating that populations with higher biomass under non-stress conditions also tended to maintain higher biomass under drought (Figure S2). Y_s was positively correlated with STI ($r = 0.90^{**}$), TOL ($r = 0.87^{**}$), MP ($r = 0.87^{**}$), and GMP ($r = 0.92^{**}$), whereas Y_p showed strong positive correlations with GMP ($r = 0.94^{**}$) and STI ($r = 0.94^{**}$) (Figure S3). These relationships indicate that STI and GMP were strongly associated with biomass performance across both non-stress and drought conditions. However, the positive correlation of TOL with Y_s should not be interpreted as evidence that higher TOL represents greater drought tolerance, because TOL measures the absolute reduction in biomass between the two conditions.

Principal component analysis explained 60.99% and 38.22% of the total variation through PC1 and PC2, respectively (Figure 2a). *T. migricus*, *T. kotschyanus-1*, *T. kotschyanus-2*, *T. pubescens*, and *T. fedtschenkoi* showed relatively high scores for both PC1 and PC2, corresponding to comparatively high biomass performance and favorable values for several drought-related indices. *T. vulgaris* showed a relatively high PC2 score, consistent with its high YSI and low SSI, indicating comparatively greater maintenance of biomass under drought relative to its non-stress performance. In contrast, *T. serpyllum* showed a relatively high PC1 score but also had the highest SSI and TOL values, reflecting high shoot dry weight under non-stress conditions accompanied by a large drought-induced reduction. *T. daenensis-1* and *T. daenensis-2* showed low PC1 and PC2 scores, consistent with their relatively low biomass production and low STI and GMP values. Overall, the multivariate

analysis revealed distinct patterns of biomass performance and drought response among populations rather than a single uniform ranking of drought tolerance (Figure 2a).

A separate cluster analysis was conducted for the thyme populations using Ward's method based on Yp, Ys, and the drought tolerance indices. The resulting dendrogram (Figure 2b) presents the populations on the horizontal axis and Euclidean distances on the vertical axis. Discriminant function analysis indicated the greatest separation when the populations were divided into four groups (Figure 2b). The first cluster, further divided into two subclusters, included *T. migricus*, *T. kotschyanus*-1, *T. kotschyanus*-2, *T. pubescens*, and *T. fedtschenkoi*. *T. vulgaris* formed the second cluster, while *T. serpyllum* formed the third cluster. *T. fallax*, *T. daenensis*-1 and *T. daenensis*-2 formed the fourth cluster. Overall, the clustering pattern was broadly consistent with the multivariate patterns observed in the PCA (Figure 2b).

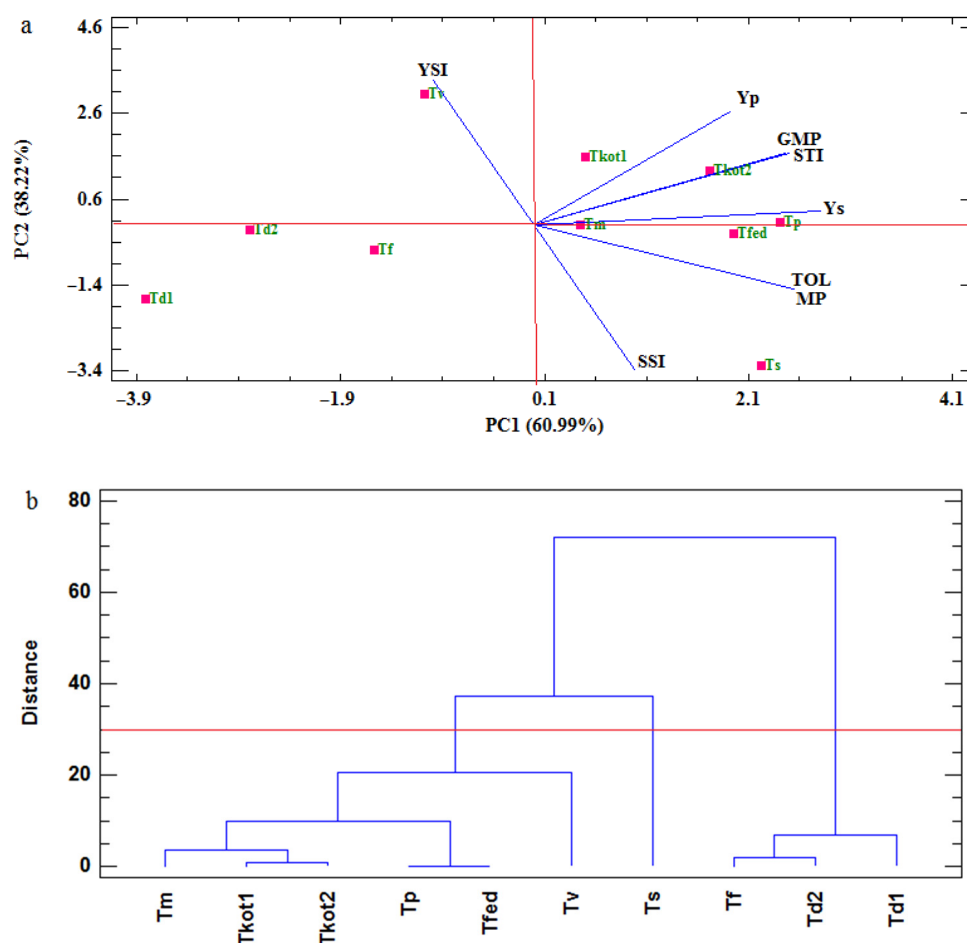


Figure 2. Principal component analysis (PCA) (a) and cluster analysis (CA) (b) of drought tolerance indices (STI, SSI, TOL, MP, GMP, and YSI) and shoot dry weight (Yp and Ys) identified drought-tolerant and drought-susceptible populations (Table S1) after application under field conditions. Yp: shoot dry weight under non-stress condition, Ys: shoot dry weight under drought stress condition, SSI: stress susceptibility index, TOL: stress tolerance, MP: mean productivity, STI: stress tolerance index, GMP: geometric mean productivity, and YSI: yield stability index. Point labels: number of complete cases, vectors: data variables. The horizontal red line represents the cut-off distance used to determine the number of clusters.

2.5. Population-Specific Effects of Drought Treatment on the Morphological, Physiological, and Biochemical Traits of Ten Thyme Populations Under Greenhouse Conditions

To complement the field experiment, a controlled greenhouse experiment was conducted to investigate the response of the ten *Thymus* populations to the same irrigation

regimes. The populations evaluated in the field experiment were also grown under greenhouse conditions.

Analysis of variance revealed significant effects of population and irrigation level on all morphological traits. Significant population $\times$ irrigation interactions were also observed for all traits except leaf width ($p \leq 0.05$), indicating that the magnitude of the morphological responses to water limitation differed among populations (Table S10).

As observed under field conditions, plant height, leaf width, and shoot fresh and dry weights generally decreased with increasing drought severity. Among the evaluated populations, *T. daenensis*-1 and *T. daenensis*-2 showed the lowest shoot fresh and dry weights, whereas *T. kotschyanus*-1 and *T. kotschyanus*-2 generally maintained higher shoot fresh and dry weights under the tested irrigation regimes. The *T. kotschyanus* populations also showed relatively smaller changes in plant height across irrigation levels (Table 2).

Overall, the magnitude of morphological responses varied among populations under greenhouse conditions. The relatively higher biomass maintained by *T. kotschyanus*-1 and *T. kotschyanus*-2, compared with *T. daenensis*-1 and *T. daenensis*-2, indicates population-dependent variation in growth responses to water limitation. These patterns were broadly comparable with those observed in the field experiment, although the magnitude of the responses differed between environments.

Analysis of variance revealed significant effects of population and irrigation level on the measured physiological traits under greenhouse conditions ($p \leq 0.05$), with population-dependent variation in the magnitude of responses (Table S11). Increasing drought severity generally reduced total chlorophyll, chlorophyll a, and chlorophyll b, whereas carotenoid content tended to increase under SWS. However, the magnitude of pigment changes varied among populations. *T. kotschyanus*-1 and *T. kotschyanus*-2 generally maintained comparatively higher chlorophyll contents across the irrigation regimes, whereas several other populations showed more pronounced declines. The chlorophyll a/b ratio also varied among populations. The highest values were observed in *T. daenensis*-1 and *T. daenensis*-2 under control conditions, whereas lower values were observed in *T. serpyllum* and *T. fedtschenkoi*. Irrigation level did not significantly affect the chlorophyll a/b ratio, indicating that the relative proportion of chlorophyll a and b was less responsive to the imposed water limitation than the individual pigment contents (Table S12). Proline concentration increased in all populations in response to increasing drought severity, although the magnitude of accumulation varied among populations (Table S13). Overall, the physiological responses differed among populations, particularly in the maintenance of photosynthetic pigments and the magnitude of proline accumulation under water limitation.

Similarly, drought stress significantly affected the measured biochemical traits of the thyme populations under greenhouse conditions ($p \leq 0.05$), with population-dependent variation in the magnitude of the responses (Table S14). Increasing drought severity generally promoted the accumulation of secondary metabolites, particularly TPC and TFC. Consistent with the field observations, *T. kotschyanus*-2 and, to a lesser extent, *T. vulgaris*, showed relatively strong increases in several biochemical traits and exhibited comparatively high TPC, TFC, and EO content under SWS. In contrast, populations such as *T. migricus* and *T. fedtschenkoi* showed smaller increases or, for some traits, reductions in metabolite contents. Antioxidant activity, as determined by the DPPH assay, increased under drought conditions in most populations, as indicated by lower IC₅₀ values (Table S15). Essential oil content also varied among populations and irrigation regimes, ranging from 0.33% to 1.47% under greenhouse conditions. Overall, the magnitude and direction of changes in phenolic compounds, antioxidant activity, and EO content differed among populations under water limitation.

Table 2. Mean comparisons of morphological traits of ten thyme populations after the application of different irrigation regimes under greenhouse conditions.

Populations	Plant Height (cm)			Internode Length (cm)			Shoot Fresh Weights (g)			Shoot Dry Weights (g)			Leaf Length (cm)			Leaf Width (cm)		
	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS	C	MWS	SWS
Tm	36 ^a	30.33 ^{a-d}	28.67 ^{b-e}	1.97 ^a	1.73 ^{a-c}	1.67 ^{a-d}	2.32 ^{c-e}	1.85 ^{f-i}	1.35 ^{k-m}	0.90 ^{i-k}	0.64 ^{k-n}	0.31 ^{o-q}	0.83 ^{gh}	0.67 ^{h-j}	0.70 ^{hi}	0.47 ^a	0.37 ^{a-d}	0.30 ^{a-d}
Tf	29 ^{b-e}	26.33 ^{c-g}	23.83 ^{d-h}	1.77 ^{ab}	1.60 ^{a-e}	1.20 ^{b-i}	2.24 ^{c-f}	1.83 ^{f-j}	1.73 ^{g-k}	0.86 ^{i-l}	0.62 ^{L-n}	0.57 ^{m-o}	0.53 ^{i-k}	0.50 ^{i-k}	0.43 ^k	0.40 ^{a-c}	0.33 ^{a-d}	0.33 ^{a-d}
Ts	30 ^{a-e}	26.83 ^{b-g}	24.33 ^{c-h}	1.17 ^{c-i}	0.87 ^{g-i}	0.77 ^{hi}	2.33 ^{c-e}	1.57 ^{h-k}	1.07 ^{L-n}	1.19 ^{f-h}	0.82 ^{j-m}	0.30 ^{o-q}	1.43 ^{a-c}	1.37 ^{a-c}	1.33 ^{a-c}	0.20 ^{b-d}	0.20 ^{b-d}	0.17 ^{cd}
Tp	31 ^{a-e}	29 ^{b-e}	26.50 ^{b-g}	1.43 ^{a-g}	1.23 ^{b-i}	1.50 ^{a-f}	3.24 ^b	2.33 ^{c-e}	2.02 ^{d-g}	2.23 ^a	1.46 ^{d-f}	0.77 ^{j-n}	1.53 ^a	1.47 ^{ab}	1.37 ^{a-c}	0.33 ^{a-d}	0.20 ^{b-d}	0.23 ^{a-d}
Td1	30 ^{a-e}	24.17 ^{c-h}	20.67 ^{gh}	1.13 ^{d-i}	1 ^{f-i}	1.17 ^{c-i}	1.40 ^{j-m}	1.03 ^{mn}	0.81 ^{no}	0.52 ^{n-p}	0.32 ^{o-q}	0.22 ^q	1.47 ^{ab}	1.43 ^{a-c}	1.27 ^{b-d}	0.40 ^{a-c}	0.33 ^{a-d}	0.23 ^{a-d}
Td2	36 ^a	28.67 ^{b-e}	20.33 ^{gh}	1.20 ^{b-i}	1 ^{f-i}	1 ^{f-i}	1.48 ^{i-l}	0.67 ^{no}	0.48 ^o	0.69 ^{k-n}	0.26 ^{pq}	0.20 ^q	1.47 ^{ab}	1.33 ^{a-c}	1.23 ^{c-e}	0.33 ^{a-d}	0.27 ^{a-d}	0.20 ^{b-d}
Tfed	26.33 ^{c-g}	23.83 ^{d-h}	18.67 ^h	1.07 ^{e-i}	1 ^{f-i}	1.03 ^{e-i}	1.97 ^{e-h}	1.68 ^{g-k}	1.38 ^{k-m}	1.28 ^{e-g}	1.13 ^{h-i}	0.98 ^{h-j}	1.03 ^{e-g}	1.03 ^{e-g}	0.93 ^{fg}	0.13 ^d	0.37 ^{a-d}	0.23 ^{a-d}
Tkot1	26.50 ^{b-g}	23.83 ^{d-h}	27.67 ^{b-f}	0.67 ⁱ	1.27 ^{b-h}	1.23 ^{b-i}	3.80 ^a	2.43 ^{cd}	1.48 ^{i-m}	1.49 ^{de}	1.29 ^{e-g}	0.80 ^{j-m}	0.83 ^{gh}	0.93 ^{fg}	0.83 ^{gh}	0.37 ^{a-d}	0.33 ^{a-d}	0.30 ^{a-d}
Tkot2	26.67 ^{b-g}	18.67 ^g	21.67 ^{f-h}	1.40 ^{a-g}	1.40 ^{a-g}	1.17 ^{c-i}	4.23 ^a	3.23 ^b	1.98 ^{d-h}	2.03 ^{ab}	1.65 ^{cd}	1.18 ^{f-h}	0.97 ^{fg}	1.10 ^{d-f}	1.10 ^{d-f}	0.43 ^{ab}	0.33 ^{a-d}	0.30 ^{a-d}
Tv	33.33 ^{ab}	23.33 ^{e-h}	18 ^h	1.57 ^{a-f}	1.20 ^{b-i}	1.37 ^{b-g}	3.30 ^b	2.59 ^c	2.30 ^{c-e}	1.88 ^{bc}	1.09 ^{h-i}	1.03 ^{h-j}	0.63 ^{h-k}	0.60 ^{i-k}	0.47 ^{jk}	0.30 ^{a-d}	0.30 ^{a-d}	0.23 ^{a-d}

C, control; MWS, moderate water stress; SWS, severe water stress. Tm: *T. migricus*, Tf: *T. fallax*, Ts: *T. serpyllum*, Tp: *T. pubescens*, Td1: *T. daenensis-1*, Td2: *T. daenensis-2*, Tfed: *T. fedtschenkoi*, Tkot1: *T. kotschyanus-1*, Tkot2: *T. kotschyanus-2*, and Tv: *T. vulgaris*. *n* = 3, the letters are based on least significant difference (LSD) test at the 5% probability level.

PCA of the physiological and biochemical traits revealed distinct multivariate patterns among the ten thyme populations under different irrigation regimes (Figure 3). Under control conditions, PC1 was positively associated with chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents, whereas PC2 was primarily associated with DPPH activity (Figure 3a). Under MWS, PC1 was positively associated with EL, proline, and RWC, while TFC and TPC showed stronger associations with PC2 (Figure 3b). Under SWS, PC1 was positively associated with chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, whereas DPPH activity and EO content were associated with PC2 (Figure 3c).

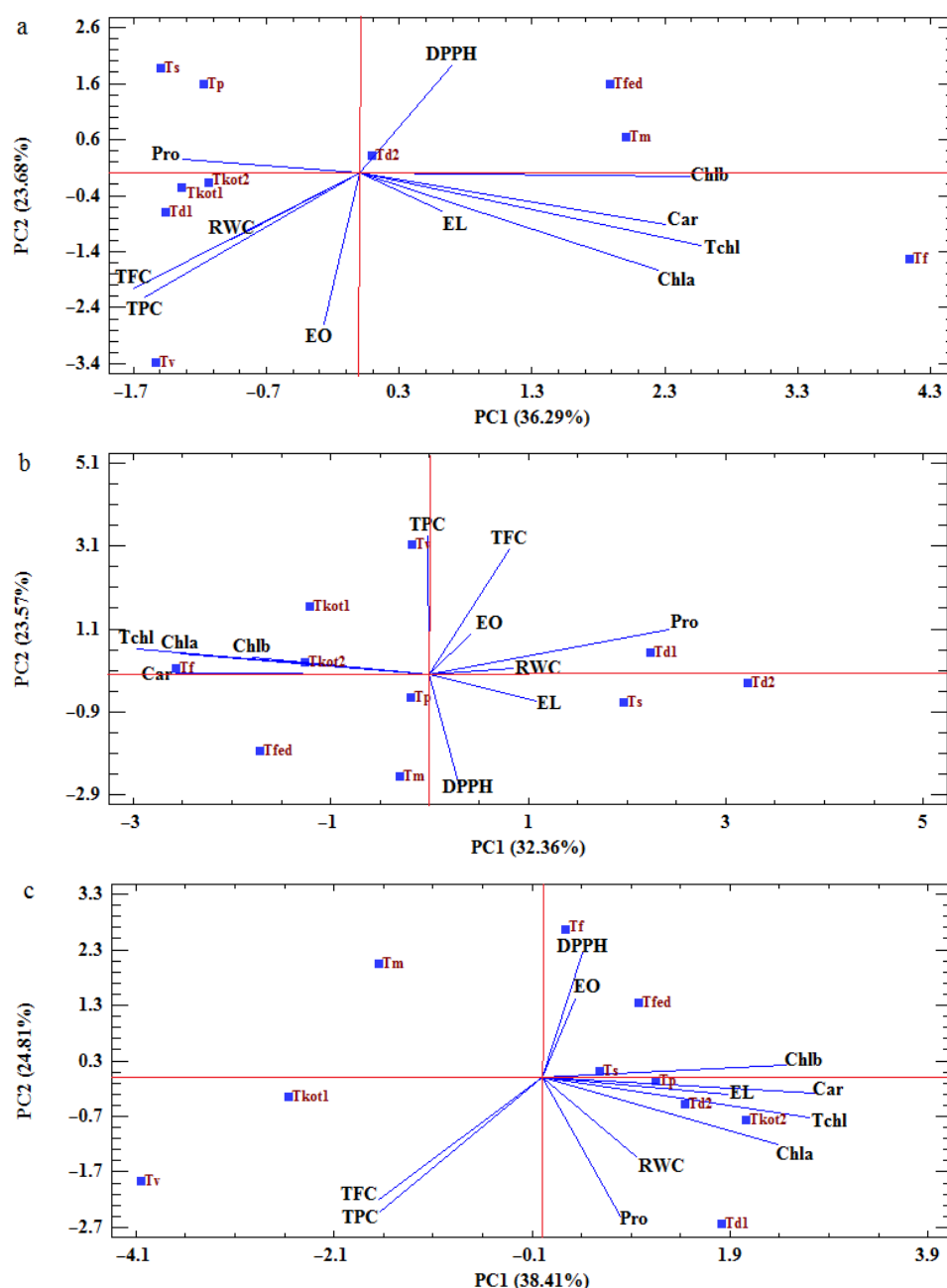


Figure 3. Principal component analysis (PCA) based on the biochemical and physiological traits of ten thyme populations (Table S1) after the application of different irrigation regimes: (a) control, (b) moderate water stress, and (c) severe water stress under greenhouse conditions. TPC: total phenols, TFC: total flavonoids, EO: essential oil, EL: electrolyte leakage, RWC: relative water content, Pro: proline, Chla: chlorophyll a, Chlb: chlorophyll b, Tchl: total chlorophyll, and Car: carotenoids. Point labels: number of complete cases, vectors: data variables.

The PCA biplots also revealed similarities in the multivariate trait profiles of some populations across irrigation regimes. Under control and stress conditions, *T. migricus*, *T. fedtschenkoi*, and *T. fallax* occupied relatively similar positions in the PCA space. Likewise, *T. serpyllum*, *T. daenensis-1*, and *T. daenensis-2* showed relatively close positions under control and MWS conditions, while *T. kotschyanus-1* and *T. kotschyanus-2* also showed similar multivariate patterns under these conditions. Other populations occupied more distinct positions in the PCA space (Figure 3). Overall, the multivariate patterns observed under greenhouse conditions differed from those observed in the field experiment, indicating that the relative contribution of physiological and biochemical traits to population separation varied between the two experimental environments.

We also analyzed drought-response indicators in the greenhouse experiment. Analysis of variance revealed significant differences ($p \leq 0.05$) among the thyme populations in shoot dry weight under non-stress (Y_p) and drought-stress (Y_s) conditions and in most drought tolerance indices, whereas SSI and YSI did not differ significantly among populations (Table S16). *T. pubescens* showed the highest shoot dry weight under non-stress conditions, whereas *T. kotschyanus-2* exhibited the highest Y_s (Table S17). STI was highest in *T. kotschyanus-2*, followed by *T. vulgaris* and *T. pubescens*, whereas *T. daenensis-1* and *T. daenensis-2* showed the lowest STI values (Table S17). GMP showed a similar pattern, with the highest value in *T. kotschyanus-2*, followed by *T. vulgaris* and *T. pubescens*. YSI was highest in *T. fedtschenkoi* and *T. fallax*, indicating a greater proportion of shoot dry weight maintained under drought relative to non-stress conditions, whereas *T. serpyllum* had the lowest YSI (Table S17). SSI was highest in *T. serpyllum*, followed by *T. daenensis-2*, *T. pubescens*, and *T. migricus*, indicating greater relative susceptibility according to this index. TOL was highest in *T. pubescens*, reflecting the largest absolute reduction in shoot dry weight between non-stress and drought conditions. Overall, the indices provided complementary information on drought response, with *T. kotschyanus-2* showing relatively favorable values for several performance-related indices, whereas *T. serpyllum* showed a comparatively high susceptibility profile based on its high SSI and low YSI values (Table S17).

Greenhouse analyses revealed a significant positive correlation between shoot dry weight under drought stress (Y_s) and non-stress conditions (Y_p), indicating that populations with higher shoot dry weight under non-stress conditions also tended to maintain higher biomass under drought. Correlation analysis showed that Y_s was strongly associated with STI, YSI, and GMP, whereas Y_p was positively correlated with GMP, MP, TOL, and STI. These relationships indicate that the drought tolerance indices captured different aspects of biomass performance and drought response and should therefore be interpreted as complementary rather than as providing a single ranking of drought tolerance (Figure S3).

PCA biplot analysis explained 60.75% and 37.91% of the total variation through PC1 and PC2, respectively (Figure 4a). PC1 was positively associated with Y_p , Y_s , STI, MP, and GMP, distinguishing populations with relatively higher biomass performance under both non-stress and drought conditions. PC2 showed positive associations with Y_p , STI, TOL, and MP and negative associations with Y_s , GMP, and YSI, reflecting additional variation in drought-response traits. *T. pubescens* and *T. kotschyanus-2* showed relatively high PC1 scores, whereas *T. serpyllum*, *T. fallax*, *T. daenensis-1*, and *T. daenensis-2* showed comparatively lower scores. Overall, PCA revealed distinct multivariate patterns of biomass performance and drought response rather than a single ranking of drought tolerance (Figure 4a). Cluster analysis using Ward's method based on Y_p , Y_s , and drought tolerance indices showed a broadly consistent grouping pattern (Figure 4b).

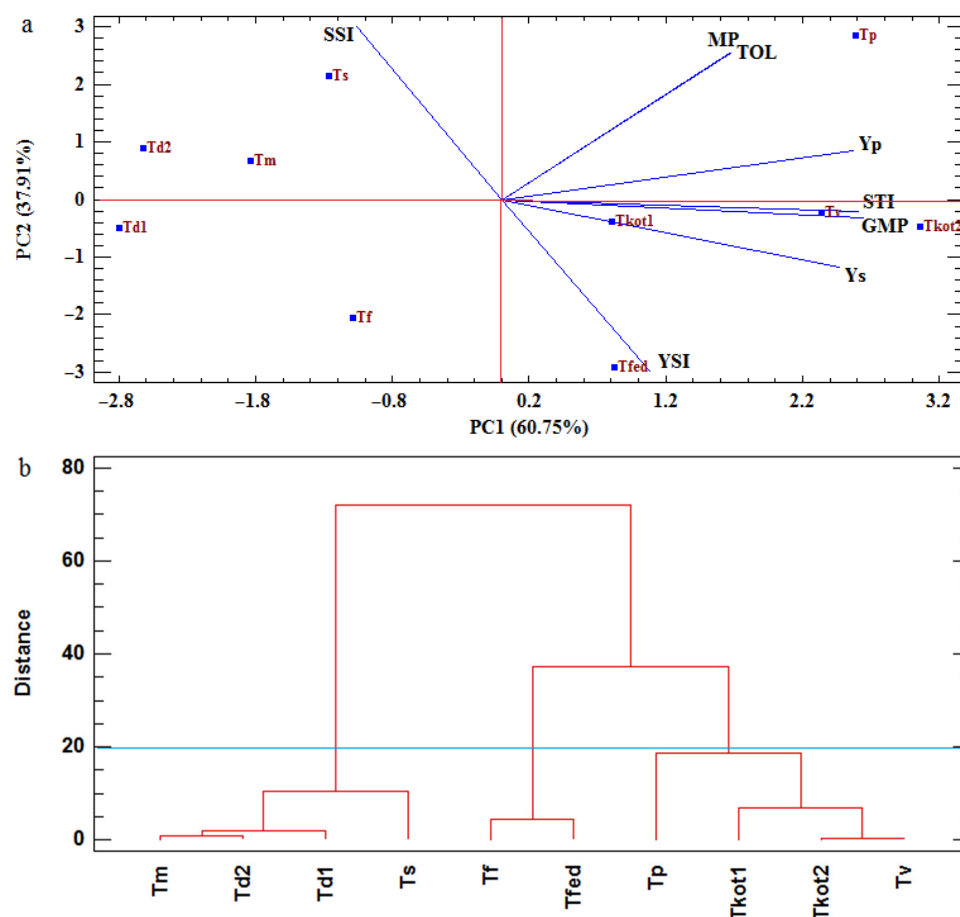


Figure 4. Principal component analysis (PCA) (a) and cluster analysis (CA) (b) of drought tolerance indices (STI, SSI, TOL, MP, GMP, and YSI) and shoot dry weight (Yp and Ys) identified drought-tolerant and drought-susceptible populations (Table S1) under greenhouse conditions. Yp: shoot dry weight under non-stress condition, Ys: shoot dry weight under drought stress condition, SSI: stress susceptibility index, TOL: stress tolerance, MP: mean productivity, STI: stress tolerance index, GMP: geometric mean productivity, and YSI: yield stability index. Point labels: number of complete cases, vectors: data variables. The horizontal blue line represents the cut-off distance used to determine the number of clusters.

2.6. Effect of Drought Stress on Specific Phenolic Compounds in Three Selected *Thymus* Populations Under Field and Greenhouse Conditions

Three thymus populations representing contrasting drought-response profiles were selected for targeted HPLC analysis based on their morphological, physiological, biochemical and drought tolerance indices results. The objective of this analysis was to further characterize population-dependent variation in specific phenolic compounds under different irrigation regimes and to examine whether populations showing relatively better drought performance also exhibited distinct phenolic profiles. Fourteen phenolic compounds were identified and quantified in the selected populations under both field and greenhouse conditions, including four flavonoids (apigenin, luteolin, rutin, and quercetin) and ten phenolic acids (gallic acid, chlorogenic acid, caffeic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, ellagic acid, *p*-coumaric acid, ferulic acid, and rosmarinic acid) (Figures S5 and S6). Representative HPLC chromatograms of the analyzed samples are provided in Supplementary Figure S4. The occurrence and abundance of individual compounds varied among populations and irrigation regimes. For example, caffeic acid was detected only in *T. serpyllum*, whereas 4-hydroxybenzoic acid was detected in *T. vulgaris* and *T. serpyllum*. Ellagic acid was detected in *T. vulgaris* under field conditions and in

both *T. vulgaris* and *T. serpyllum* under greenhouse conditions. Quercetin was detected in *T. vulgaris*, whereas *p*-coumaric acid was detected in *T. serpyllum* and *T. kotschyanus-2*, and luteolin was detected in *T. vulgaris* and *T. kotschyanus-2*. Other compounds, including rosmarinic acid, ferulic acid, syringic acid, and rutin, were detected across the selected populations and generally occurred at higher concentrations (Figures S5 and S6).

Drought stress generally increased the concentrations of several phenolic compounds in the selected populations under both field and greenhouse conditions, although the magnitude and direction of responses varied among compounds and populations. Under field conditions, some compounds that were not detected or were below the detection limit under control conditions were detected under drought, including 4-hydroxybenzoic acid in *T. vulgaris*, rutin in *T. kotschyanus-2*, and apigenin in *T. serpyllum*. Under greenhouse conditions, apigenin and gallic acid were detected in *T. serpyllum* and *T. vulgaris*, respectively, under drought conditions, but were not detected or were below the detection limit under control conditions. In contrast, the concentrations of some compounds, including luteolin and quercetin, decreased under drought in certain populations (Figures S5 and S6). These results indicate that drought-induced changes in individual phenolic compounds were compound and population-dependent.

The heatmap combined with hierarchical cluster analysis (HCA) revealed differences in phenolic profiles among populations, irrigation regimes, and experimental environments (Figure 5). In several cases, field-grown and greenhouse-grown samples from the same population were separated into different clusters, such as *T. vulgaris* and *T. serpyllum* under control conditions, indicating differences in phenolic profiles between the two experimental environments. However, some samples from different environments showed relatively close clustering, such as *T. kotschyanus-2* under SWS in the greenhouse and field experiments. This pattern suggests that both population background and environmental conditions may contribute to variation in phenolic profiles. Among the measured compounds, rosmarinic acid and apigenin showed relatively similar clustering patterns, while rutin, *p*-coumaric acid and caffeic acid also showed relatively close associations in the heatmap. These associations describe similarities in their observed concentration patterns and should not be interpreted as evidence of shared biosynthetic pathways. Overall, the clustering analysis revealed population- and environment-dependent variation in phenolic profiles (Figure 5).

PCA based on the quantified phenolic compounds explained 25.03%, 21.60%, and 14.49% of the total variation through PC1, PC2, and PC3, respectively. Because the first two components accounted for less than 50% of the total variation, interpretation was based on the three-dimensional PCA representation to capture additional variation among the samples (Figure 6a,b). Several metabolites, including rosmarinic acid and apigenin, showed relatively strong associations with particular samples, including *T. kotschyanus-2* under SWS in the field and *T. serpyllum* under MWS in the greenhouse. Rutin, *p*-coumaric acid, and caffeic acid were more closely associated with others samples, including *T. vulgaris* and *T. serpyllum* under control conditions in the field. Overall, the PCA revealed distinct multivariate patterns in phenolic composition among the selected populations, irrigation regimes, and experimental environments, indicating that the accumulation of individual phenolic compounds varied according to both population and growing conditions (Figure 6a,b).

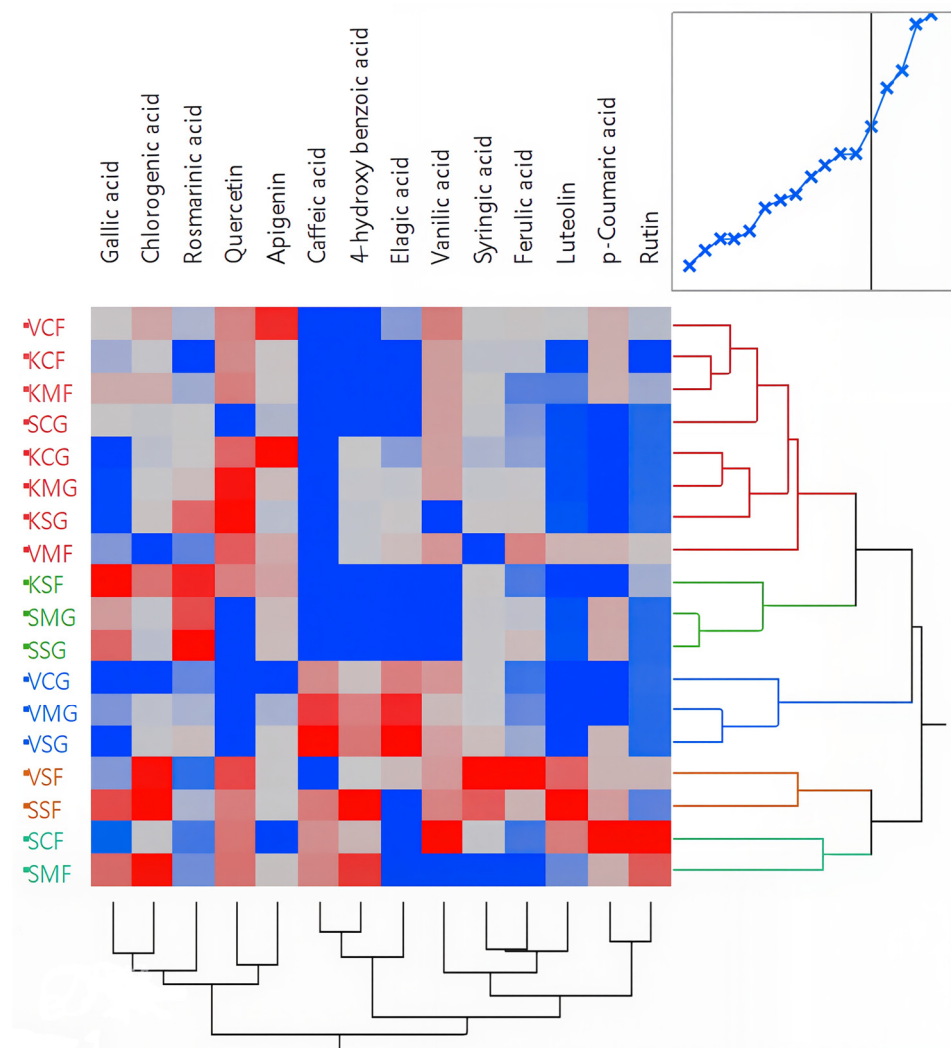


Figure 5. Hierarchically clustered heat map of different phenolic and flavonoid compounds produced by three thyme species (V, *T. vulgaris*; S, *T. serpyllum*; K, *T. kotschyanus*-2) after the application of different irrigation regimes: C, control; M, moderate water stress; and S: severe water stress under two conditions (F, field and G, greenhouse). Color intensity reflects relative trait values, with red indicating higher values, gray intermediate values, and blue lower values.

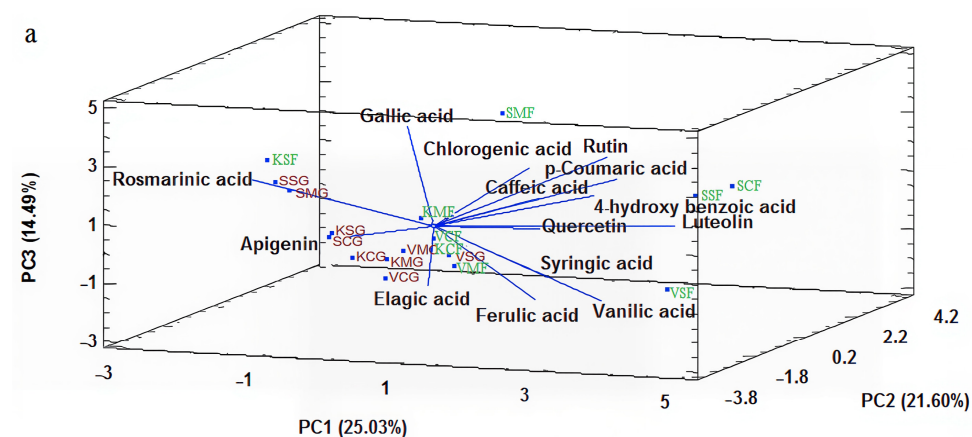


Figure 6. Cont.

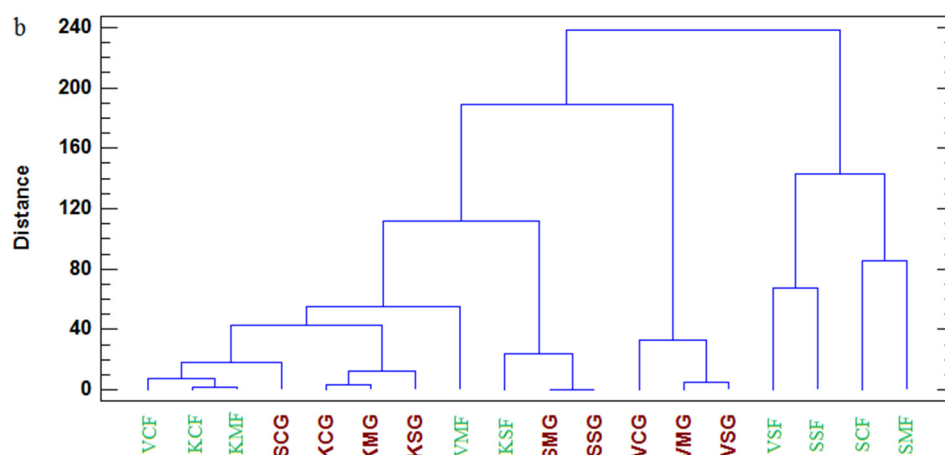


Figure 6. Component analysis (PCA) (a) and cluster analysis (CA) (b) for phenolic compounds in three thyme species (V, *T. vulgaris*; S, *T. serpyllum*; K, *T. kotschyanus*-2) after the application of different irrigation regimes (C, control; M, moderate water stress; and S, severe water stress) under two conditions (F, field (green), and G, greenhouse (red)). Point labels: number of complete cases, vectors: data variables.

3. Discussion

Drought stress is one of the most significant abiotic factors restricting the growth, productivity, and secondary metabolism of medicinal and aromatic plants, including thyme. In the present study, ten thyme populations were evaluated under greenhouse and field conditions. Drought stress was shown to result in significant changes in morphological, physiological, biochemical, and phytochemical traits. However, the intensity and direction of these responses differed substantially among populations and between environments, indicating strong population-dependent and environment-dependent variation in drought responses. These patterns highlight the complexity of drought responses in *Thymus* spp. and the value of evaluating populations under both controlled and field conditions to better characterize the consistency and context dependence of their responses.

Across both environments, drought stress reduced plant height and shoot fresh and dry weight, although populations differed in sensitivity. In the greenhouse, *T. daenensis* (Td1 and Td2) exhibited the strongest reductions in biomass, indicating comparatively greater sensitivity under field conditions; however, *T. kotschyanus*-1 and *T. kotschyanus*-2 maintained relatively stable biomass. This relative stability may reflect a greater capacity to maintain growth and tissue water status under drought stress, although the underlying mechanisms were not directly assessed in the present study. These patterns are consistent with reports on other Lamiaceae species, such as *Hyssopus officinalis*, *Salvia officinalis* and *Origanum vulgare* [28]. In contrast, *T. migricus* and *T. pubescens* produced the highest biomass under control conditions, but experienced substantial reductions under SWS, indicating that high biomass under non-stress conditions did not necessarily translate into better drought performance. The occurrence of biomass reductions in both field and greenhouse experiments highlights the general impact of water limitation on primary growth processes such as cell expansion and carbon assimilation [29]. Nevertheless, given the limited biological replication ($n = 3$), these interpretations should be viewed with caution and represent relative, not absolute, differences among populations.

Physiological traits were highly responsive to drought stress. RWC decreased consistently with increasing drought conditions, although populations differed in their ability to maintain tissue hydration. *T. vulgaris* and *T. kotschyanus*-2 maintained higher RWC values under stress, indicating better maintenance of tissue hydration under the tested conditions, while *T. serpyllum* and *T. kotschyanus*-1 experienced greater declines. Maintaining a high

RWC is a key trait associated with drought tolerance in aromatic plants [30]. Proline and EL increased in all populations under drought stress, with *T. daenensis-1* showing the highest levels. Enhanced membrane integrity under drought stress has also been associated with improved stress tolerance in other Lamiaceae members [20]. However, proline alone cannot be used as a definitive indicator of drought tolerance, as tolerance is a multivariate trait that depends on hydration status, membrane integrity, and biomass production. Therefore, to avoid overgeneralized claims, our interpretation considers RWC, EL, and biomass rather than relying on a single parameter [31].

Most populations experienced a decline in photosynthetic pigments under drought conditions; however the extent of pigment loss depended highly on population and environment. Although *T. kotschyanus-2* showed substantial reductions in some pigment traits under SWS in the field, it maintained relatively high biomass and water status and exhibited comparatively favorable responses across several physiological and biochemical traits. Therefore, its drought tolerance should not be inferred from pigment stability alone but from the integrated response of growth, water status, physiological traits, biochemical responses, and drought tolerance indices. These contrasting patterns indicate that pigment responses are population-dependent and influenced by stress severity and environmental conditions [23,32]. Previous studies have also reported reductions in chlorophyll concentration induced by drought in some thyme species [3,33] and Mexican marigold [34], while others have reported increases in chlorophyll in some cereal species under drought stress [35]. Interestingly, though the total chlorophyll content changed markedly under drought conditions, the chlorophyll a/b ratio showed significant variation among populations, yet remained unaffected by irrigation levels. Though a number of studies have shown that, under drought stress, the reduction in Chlb is greater than that of Chla, thus leading to a higher Chla/Chlb ratio [36], other studies are consistent with our results. These studies also demonstrate a population-specific decline in photosynthetic pigments that does not necessarily correspond with an increased ratio [37,38]. This suggests that, in the present study, variation in this parameter was more strongly associated with population differences than with irrigation level. Therefore, while changes in total pigments reflect the effects of stress, pigment composition (Chla/b ratio) may not reliably indicate drought resistance in all species [11,39].

Secondary metabolites and EOs increased under SWS, but the responses were population- and environment-specific, a finding also reported in other aromatic species [9]. The highest induction was observed in *T. vulgaris* and *T. kotschyanus-2*, which is consistent with enhanced TPC and TFC, as well as the highest EO content. These results are consistent with the proposed contribution of phenolic and terpenoid metabolites to antioxidant responses under drought stress [25,30]. Essential oil is one of the major groups of specialized metabolites in thyme, and both its quantity and chemical composition (e.g., thymol, carvacrol, and related monoterpenes) may vary substantially under drought and other environmental stresses [16]. Although the present study quantified EO content rather than compositional changes, previous studies indicate that stress conditions can markedly alter terpene profiles, thereby influencing both biological activity and commercial quality [17]. Such increases have been proposed to be associated with changes in leaf expansion and glandular trichome density under drought [40]. Similar responses have been reported in other aromatic plants such as *Origanum vulgare*, *Petroselinum crispum*, and *Ocimum* spp. [41–43]. Because metabolite and essential oil contents were expressed on a dry weight basis, differences in tissue water content were minimized; however, changes in biomass may still influence the total amount of metabolites produced per plant [44,45]. Drought mediated increases in EO and phenolic contents may have implications for the production of these valuable compounds, although the effects of drought severity on overall biomass and total

compound yield should also be considered [46–48]. The relatively favorable EO response observed in *T. kotschyanus*-2 in both experimental environments supports its potential for further evaluation under water-limited cultivation conditions. Conversely, populations such as *T. migricus* and *T. fedtschenkoi* showed smaller increases or even reductions. This is consistent with previous studies and may indicate a comparatively weaker induction of the measured antioxidant related traits under the tested conditions [18,34,49,50]. However, comparisons between greenhouse and field trends should be interpreted with caution due to differences in environmental conditions, including light intensity and UV-B exposure, which can independently modulate phenylpropanoid and terpene pathways [51]. Thus, while patterns were broadly consistent, environment-specific metabolic shifts underscore the absence of full cross-environment equivalence.

The differences in the proportions and composition of secondary metabolites and essential oils among *Thymus* species and populations may partly reflect genetic variation in their metabolic capacities. Considerable chemical polymorphism has been documented within the genus *Thymus*, with distinct populations and species exhibiting different essential oil profiles and chemotypes characterized by variation in major constituents such as thymol, carvacrol, linalool, and related terpenoids [52]. Molecular studies have also demonstrated associations between genetic differentiation and chemotypic variation in *Thymus*, supporting a genetic contribution to differences in essential oil composition [52,53]. However, environmental conditions, geographical origin, and developmental factors can also strongly modify metabolite accumulation and essential oil composition. Thus, the population-dependent differences observed in the present study may reflect the combined influence of population background and environmental conditions rather than genetic variation alone [54].

PCA revealed that, under control conditions, photosynthetic pigments contributed most to population separation; under SWS, however, phenolics, flavonoids, and EO content dominated the variance. PCA separated populations exhibiting relatively higher drought performance, including *T. pubescens*, *T. kotschyanus*-1, and *T. kotschyanus*-2, from populations showing comparatively poorer performance under drought, including *T. serpyllum*, *T. fallax*, *T. daenensis*-1, and *T. daenensis*-2. However, these groupings represent multivariate patterns of trait variation rather than definitive classification of drought tolerance. PCA also showed that biochemical traits (phenolics, EO, and antioxidant activity) played a greater role in population separation under greenhouse drought than under field conditions. This suggests that biochemical traits contributed more strongly to population separation under the controlled greenhouse conditions used in this study. The differences observed between field and greenhouse conditions may partly reflect differences in environmental factors, including light intensity and UV-B exposure, which were not directly quantified in the present study. Drought and UV-B light may affect plants in different ways, and the combination of these two stresses can induce antagonistic, additive, or synergistic responses compared to a single stress factor [51].

Correlation analyses and drought tolerance indices (STI, GMP, YSI, and TOL) provided additional support for population-level differences. *T. kotschyanus*-2 and *T. pubescens* generally showed relatively favorable values for several performance-related indices, whereas *T. daenensis*-1 and *T. serpyllum* showed less favorable index profiles. Cluster analysis further separated populations according to their multivariate patterns of drought-related traits. *T. kotschyanus*-1 showed an intermediate drought-response profile, whereas *T. fedtschenkoi* maintained relatively favorable responses for some traits but had lower shoot dry weight potential. The drought tolerance indices provided complementary rather than identical information on population responses to water stress. STI and GMP reflect the ability to maintain relatively high biomass under both non-stress and stress conditions, whereas TOL

represents the absolute reduction in biomass, SSI reflects relative susceptibility to stress, and YSI represents the proportion of biomass maintained under stress. Consequently, contrasting rankings among these indices do not necessarily indicate inconsistency, but rather reflect their different mathematical and biological interpretations. These findings are consistent with previous studies suggesting that STI and GMP can be useful indicators for identifying populations with relatively high performance under both stressed and non-stressed conditions [19,55]. However, these indices should be interpreted independently within each environment, as no combined statistical model was used to integrate greenhouse and field data. Therefore, drought tolerance in the present study was not inferred from a single index, but was evaluated by considering the drought tolerance indices together with biomass and physiological and biochemical responses.

Targeted phenolic profiling of the three selected populations revealed that rosmarinic acid was the dominant compound, increasing most strongly under drought conditions, particularly in *T. kotschyanus*-2. The consistent dominance of rosmarinic acid is in agreement with previous studies on thyme and related species [3,17,56]. Population-specific patterns were evident for other phenolic compounds, such as rutin, *p*-coumaric acid, and luteolin. These results indicate population-dependent differences in phenolic responses among the three selected populations, with the magnitude of these responses varying between the two experimental environments [57,58]. The increased accumulation of compounds such as gallic acid and apigenin may reflect shifts in phenolic metabolism under drought stress [59]. However, given the absence of a formal population $\times$ environment ($P \times E$) statistical model, these interpretations should be considered descriptive rather than inferential. Although repeated trends were observed in both environments, the underlying $P \times E$ structure remains unresolved, representing a limitation of the current study.

Overall, greenhouse experiments provided greater insight into biochemical mechanisms, whereas field experiments captured environmental variability. Shared trends, such as reduced biomass and photosynthetic pigments, increased proline and EL, and enhanced phenolic and EO content, were observed across both environments. However, differences in magnitude emphasize that the two environments should not be considered directly comparable. The lack of a unified statistical model prohibits quantitative cross-environment comparison; therefore, our conclusions remain environment-specific. Future studies should incorporate formal $G \times E$ analytical frameworks, multi-environment and multi-year trials, and molecular approaches to investigate the mechanisms underlying population-specific drought responses. Taken together, our findings provide an integrated, yet context-dependent, view of drought responses among thyme populations. Although consistent physiological and biochemical patterns emerged, the interpretation of drought tolerance is necessarily limited by environmental heterogeneity, limited replication, and the absence of a formal $G \times E$ model. Therefore, the scope of our conclusions is limited to relative performance within each experimental environment.

4. Materials and Methods

4.1. Plant Material and Growing Conditions

This study examined ten thyme populations (eight species) from Iran and *T. vulgaris* as a reference from Europe (Table S1). Four species (*T. migricus*, *T. fallax*, *T. serpyllum*, and *T. pubescens*) were collected from the same multispecies natural habitat in Shahedieh (Yazd Province), where these taxa naturally grow in sympatry. The remaining Iranian populations were sampled from distinct geographic regions across the country, each representing a unique ecological background. For species represented by more than one accession, such as *T. daenensis* and *T. kotschyanus*, two independent populations were collected from different geographic locations. These populations were labeled “1” and “2” to distinguish their

collection sites and genetic backgrounds. All population origins and accession codes are provided in Table S1. These populations were selected because they represent native Iranian *Thymus* taxa that naturally occur across diverse ecological regions. The seeds were provided by the Research Institute of Forests and Rangeland in Tehran, Iran. Species were identified by Prof. Mozaffarian using Flora Iranica [14]. Voucher specimens were deposited in the Research Institute of Forests and Rangeland Herbarium in Tehran, Iran. This research was conducted in two consecutive phases: a field experiment followed by a greenhouse experiment.

4.1.1. Field Experiment

The seeds were germinated in seedling plastic pots containing a 1:1 blend of peat moss and coco peat. The pots were placed in a greenhouse with an average temperature of 25 °C and 50% humidity for two months. The seedlings were then transplanted into the field. The field experiment was conducted as a split-plot experiment within a randomized complete block design (RCBD), with *Thymus* populations as the main-plot factor and irrigation regime as the subplot factor. Three blocks were used, and all populations were represented in each block. The three irrigation regimes were randomized within each population main plot. Each subplot (biological replicate) consisted of 10 plants, and the mean value of the 10 plants was used as the experimental unit for statistical analysis. The main effect of population was tested against the main-plot error (block $\times$ population), whereas irrigation and the population $\times$ irrigation interaction were tested against the subplot residual error. Each plot included 6 rows, each 2 m long and 50 cm apart, with individual plants spaced 30 cm apart.

The research farm was located 32°38' north and 51°39' east. The average annual rainfall and minimum and maximum absolute temperatures were 122.8 mm, 9.1 °C, and 23.4 °C. The soil texture in the field was clay loam with a bulk density of 1.4 g cm⁻³ and a pH of 7.8.

Soil samples were collected before irrigation from three soil depths of 0–15, 15–30, and 30–45 cm to quantify the soil water content and calculate irrigation water content based on a 45 cm rooting depth. The irrigation depth was approximated using the following formula:

$$I = [(FC - \theta)/100] \times D \times B \quad (1)$$

where *I* is the irrigation depth (cm), *FC* [−0.03 MPa] is the soil gravimetric moisture at field capacity (22%), θ [−1.5 MPa] is soil gravimetric moisture percentage at irrigation time (10%), *D* is the root-zone depth (45 cm), and *B* is the soil bulk density of the soil in the root zone (1.4 g cm⁻³) [51]. Irrigation began at transplanting time and continued weekly until the plants were well-established in the field and before flowering (vegetative development).

The irrigation treatments were 100%, 70%, and 40% of field capacity (FC), equaling the control (C), moderate water stress (MWS), and severe water stress (SWS), respectively. The amount of irrigation water applied at each event was calculated based on soil water depletion. The net irrigation depth (*I_d*) was estimated as the product of the irrigation requirement (*I*) and the allowable depletion fraction of soil moisture (*p*). The gross irrigation depth (*I_g*) was then obtained by adjusting for irrigation efficiency using the equation $I_g = (I_d/E_a) \times 100$, where the irrigation efficiency (*E_a*) was assumed to be 65%. Water was supplied through a pressurized irrigation system with a controlled discharge rate. Field operations and irrigation management followed the standard agronomic procedures reported in the literature [49,50].

4.1.2. Greenhouse Experiment

The seeds were germinated in seedling plastic pots containing a 1:1 blend of peat moss and coco peat. The pots were placed in a greenhouse with an average temperature of 25 °C, 60% relative humidity under natural daylight, average photosynthetic photon flux density (PPFD) of approximately 450–600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at canopy level and a 16/8 h light/dark photoperiod. After two weeks, the seedlings were transplanted into plastic pots (30 cm in diameter and 30 cm in height) filled with a 2:1:1 ratio of sieved field soil, sand and leaf mold, resulting in a well-drained sandy loam texture. Each pot contained 5.8 kg of the prepared growth medium. Throughout the experiment, no fertilizers or additional nutrients were used to ensure that all observed effects were solely caused by drought stress. Each treatment included three biological replicates (each biological replicate consisted of three pots, each with five plants, and the mean of each replicate was considered the experimental unit for statistical analysis), and all biochemical analyses were conducted in triplicate (technical replicates). After two months, irrigation treatments were applied for fifty days.

The greenhouse irrigation regimes corresponded to 100%, 70%, and 40% of field capacity (FC), which was identical to those applied in the field. The amount of water required for each pot was determined by estimating the soil's field capacity (FC) at the beginning of the experiment using a gravimetric (pot-weighing) approach [60]. Pots were filled with the soil mixture and irrigated to full saturation. To minimize evaporative losses, each pot was covered with plastic film and allowed to drain freely. After 24 h, the weight of each pot was recorded at regular intervals until a constant weight was reached, indicating the field capacity. Then, soil samples were collected to determine the fresh and dry weights. The soil moisture percentage at field capacity (FC) was calculated as follows:

$$\text{Soil water content (\%)} = (\text{soil fresh weight} - \text{soil dry weight}) / \text{soil dry weight} \times 100 \quad (2)$$

Based on the water retained at FC, target weights corresponding to 100%, 70%, and 40% FC were established. During the experiment, the pots were weighed daily and the required amount of water was added to restore each treatment to its designated weight. This experiment solely examined vegetative development in thyme populations. Sampling was done after 50 days of irrigation treatment.

4.2. Growth Parameters

Growth measurements were taken at the end of the experiment, which was almost 50 days after the start of the drought stress treatment. These measurements included plant height (cm), leaf length (cm), leaf width (cm), internode length (cm), and shoot fresh and dry weight (aerial parts) (g).

4.3. Measurement of Drought Resistance Indices

Drought tolerance indices were calculated to characterize different aspects of population performance under water stress. Because these indices represent different components of drought response, they were interpreted individually and in combination with shoot dry biomass rather than being used as independent criteria for assigning a single drought-tolerance category. Mean productivity (MP) [61], stress tolerance index (STI) [62], geometric mean productivity (GMP) [62], tolerance index (TOL) [61], stress susceptibility index (SSI) [63], and yield stability index (YSI) [64] were estimated for each population based on shoot dry weight under stress (Y_s) and shoot dry weight non-stress (Y_p) conditions to

select populations further. Quantitative drought resistance indices were calculated using the following formulas:

$$MP = \frac{Y_p + Y_s}{2} \quad (3)$$

$$STI = \frac{Y_s \times Y_p}{\bar{Y}_p^2} \quad (4)$$

$$GMP = \sqrt{Y_p \times Y_s} \quad (5)$$

$$ToL = Y_p - Y_s \quad (6)$$

$$SSI = \frac{1 - (Y_s \div Y_p)}{1 - (\bar{Y}_s \div \bar{Y}_p)} \quad (7)$$

$$YSI = \frac{Y_s}{Y_p} \quad (8)$$

where Y_s is the shoot dry weight of a population under severe water stress (40% of field capacity) and Y_p is the shoot dry weight for a population under control conditions (100% of field capacity); $\bar{Y}_s$ is the mean shoot dry weight of all populations under stress; $\bar{Y}_p$ is the mean shoot dry weight of populations under non-stress treatments.

4.4. Essential Oil (EO) Extraction

The essential oil content was determined following the European Pharmacopeia method (ver. 8.2, monograph 2.8.12). Thyme plants were shade-dried at room temperature (20–25 °C) for seven days. Then, 50 g samples of the dried aerial parts were subjected to hydro-distillation for 6 h using Clevenger equipment. The obtained EO was dried over anhydrous sodium sulfate, measured based on dry matter, and stored at 4 °C until analysis [65].

4.5. Determination of Proline Content

The proline concentration was estimated following the Bates et al. [66] procedure, with slight modifications. Fresh leaf samples (0.2 g) were homogenized in 10 mL of 3% (*w/v*) sulfosalicylic acid and kept at 4 °C. The homogenate was centrifuged at 5000 rpm for 20 min. The resulting supernatant was collected for analysis. A 2 mL aliquot of the extract was mixed with 2 mL of 2.5% (*w/v*) ninhydrin reagent, 2 mL of 60% (*v/v*) phosphoric acid, and 1 mL of glacial acetic acid. The mixture was incubated in a boiling water bath for 1 h and then rapidly cooled in an ice bath. The resulting chromophore was extracted with 4 mL of toluene. The absorbance of the toluene phase was then measured at 518 nm using a UV-160A photometer (Shimadzu, Kyoto, Japan). Proline concentration was determined using a standard curve prepared with L-proline (Sigma-Aldrich, Burlington, MA, USA; ≥98% purity) and expressed as $\mu\text{mol g}^{-1}$ fresh weight.

4.6. Determination of Relative Water Content (RWC)

Relative water content was measured according to Smart and Bingham [67]. Small leaf segments were excised from healthy, fully expanded leaves, and their fresh weight (FW) was immediately determined using a digital balance. To achieve full turgidity, the samples were immersed in distilled water in plastic cups. They were kept at room temperature (approximately 23 °C) in the dark for 24 h. After incubation, the leaf segments were gently blotted with filter paper to remove surface moisture, and their turgid weight (TW) was

measured. The samples were subsequently oven-dried at 72 °C for 48 h to obtain the dry weight (DW). The RWC of the leaves was calculated using the following equation:

$$\text{RWC}(\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100 \quad (9)$$

where FW is the fresh weight, TW is the turgid weight, and DW is the dry weight of the leaf tissue.

4.7. Determination of Electrolyte Leakage (EL)

Electrolyte leakage was determined using the method described by Barranco and Ruiz [68] with slight modifications. Fresh leaf discs (approximately 0.2 g) were cut from fully expanded leaves and gently rinsed with deionized water to remove any surface-adhered ions. The discs were then placed in test tubes containing 10 mL of deionized water and incubated at room temperature for 24 h. After incubation, the initial electrical conductivity (EC_1) of the bathing solution was measured using a conductivity meter. Subsequently, the same tubes were heated in boiling water for 10–15 min to disrupt the cell membranes completely and release all electrolytes. After cooling the samples to room temperature, the final electrical conductivity (EC_2) was measured. EL was calculated as the percentage ratio of the initial to the final electrical conductivity values, according to the following formula:

$$\text{EL}(\%) = \text{EC}_1 / \text{EC}_2 \times 100 \quad (10)$$

4.8. Determination of Photosynthetic Pigments Content

The content of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) was determined using spectrophotometry after extracting fresh leaves (0.2 g FW) with 20 mL 80% (v/v) of acetone. The samples were homogenized, briefly sonicated (30 s), and centrifuged at $10,000 \times g$ for 10 min. The absorbance of the supernatant was measured at 663.2, 646.8, and 470 nm using a UV-160A photometer (Shimadzu, Kyoto, Japan). Pigment concentrations were calculated according to Lichtenthaler and Buschmann [69] using the following equations:

$$\text{Chlorophyll a (mg L}^{-1}\text{)} = 12.25 A_{663.2} - 2.79 A_{646.8} \quad (11)$$

$$\text{Chlorophyll b (mg L}^{-1}\text{)} = 21.50 A_{646.8} - 5.10 A_{663.2} \quad (12)$$

$$\text{Total chlorophyll} = \text{Chl a} + \text{Chl b} \quad (13)$$

$$\text{Carotenoids (mg L}^{-1}\text{)} = (1000 A_{470} - 1.82 \text{ Chl a} - 85.02 \text{ Chl b}) / 198 \quad (14)$$

Final values were expressed as mg g^{-1} fresh weight. All measurements were performed in duplicate.

4.9. Measurement of Total Phenolic Content (TPC)

Phenolic compounds were determined based on the Folin–Ciocalteu method described by Ardestani and Yazdanparast [70]. To prepare a sample extract, 250 mg of dried-milled sample was mixed with 10 mL of 80% methanol, shaken thoroughly, and filtered; then, 0.5 mL of the resulting plant extract was mixed with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent. After 5 min, 2 mL of 7.5% (w/v) sodium carbonate solution was added to the mixture. The reaction mixture was incubated in a water bath for 45 min. The absorbance of the resulting blue-colored solution was measured at 765 nm using a UV-160A photometer (Shimadzu, Kyoto, Japan). Tannic acid was used as the reference standard, and TPC was calculated as $\text{mg of tannic acid equivalents (TAEs) g}^{-1}$ dry weight.

4.10. Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was adapted for the determination of total flavonoids [71]. For the assay, 250 μL of plant extract was mixed with 75 μL of 5% (w/v) sodium nitrite (NaNO_2) solution and allowed to stand for 5 min. Then, 150 μL of 10% (w/v) aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) solution was added, followed by incubation for another 5 min. Subsequently, 500 μL of 1 M sodium hydroxide (NaOH) and 2.5 mL of distilled water were added to the reaction mixture. After 15 min at room temperature, the absorbance was measured at 510 nm against a reagent blank using a UV-160A photometer (Shimadzu, Kyoto, Japan). Quercetin was used as the standard to prepare calibration curves, and TFC was calculated in mg of quercetin equivalents (QE) g^{-1} dry weight.

4.11. DPPH Radical Scavenging Assay

Antioxidant capacity was evaluated by measuring DPPH radical scavenging activity according to Ang et al. [72], with slight modifications. In brief, 0.1 mL of thyme extract at different concentrations (50, 100, and 300 $\mu\text{g/mL}$) was added to 5 mL of a 0.1 mM DPPH solution prepared in methanol. The reaction mixtures were incubated at 25 $^\circ\text{C}$ for 30 min with gentle shaking prior to absorbance measurement. Absorbance of the samples was measured at 517 nm using a UV-160A photometer (Shimadzu, Kyoto, Japan), with a control consisting of 0.1 mL of 80% (v/v) methanol and 5 mL of DPPH solution. The percentage of inhibition of the samples was estimated using the following formula:

$$\% \text{ Inhibition} = \frac{AB - AA}{AB} \times 100 \quad (15)$$

where AB is the absorbance of the control and AA is the absorbance of the sample. Then, the inhibition percentage was plotted against the extract concentration, and 50% of the inhibitory concentration (IC_{50}) of the DPPH values was calculated.

4.12. High-Performance Liquid Chromatography (HPLC)

Three *Thymus* species were selected based on the aforementioned drought resistance indices (see the Results Section), and their phenolic compounds were measured in leaves of plants grown in the field and glasshouse under the three irrigation regimes. For phenolic analysis, 250 mg of oven-dried leaf material was extracted with 25 mL of 80% (v/v) methanol and agitated at 25 $^\circ\text{C}$ for 24 h. The resulting extracts were evaporated to dryness, reconstituted in 1 mL of HPLC solvent A, and passed through a 0.22 μm membrane filter prior to analysis. A 20 μL aliquot was injected into an Agilent 1090 HPLC system, and compounds were monitored at 260 and 350 nm. Chromatographic separation and operating conditions followed the method described by Nouraei et al. [73]. External standards for chromatography were solutions of pure known compounds that were available for this study. Stock standard solutions were prepared in methanol and working standards were prepared by serial dilution to generate a calibration range of 1–200 $\mu\text{g mL}^{-1}$. The stationary phase consisted of a 250 mm $\times$ 4.6 mm (5 μm) Symmetric C18 column (Waters Corp., Milford, MA, USA) with a 10 mm $\times$ 4 mm I.D., 5 μm guard column. Chromatographic separation was performed using a binary solvent system consisting of solvents A and B at a constant flow rate of 0.8 mL min^{-1} . The column temperature was maintained at 25 $^\circ\text{C}$, and detection was carried out with a UV detector over the wavelength range of 200–400 nm. Solvent A consisted of water containing 0.1% formic acid, whereas solvent B was acetonitrile supplemented with 0.1% formic acid. Elution was achieved using a gradient program starting from 10% and increasing to 26% B over 40 min, followed by an increase to 65% B at 70 min, and finally reaching 100% B at 75 min. Calibration curves were generated for each standard (three or more concentrations). The regression coefficients (r^2)

for all calibration curves were ≥ 0.985 . The limits of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio (3:1 for LOD; 10:1 for LOQ). Method repeatability was assessed by repeatedly injecting ($n = 6$) a mid-range standard and a sample; the relative standard deviation (RSD) was $< 5\%$ for retention time and $< 7\%$ for peak area. Full calibration plots for all standards are included as Supplementary Figure S1A–N. Phenolic compounds were calculated by comparing the peak areas to their retention times and UV spectra with pure standards (Phytolab, Vestenbergsgreuth, Germany, purity greater than 98%). Finally, the results are presented as $\text{mg } 100 \text{ g}^{-1}$ of sample dry weight.

4.13. Statistical Analysis

The field experiment was conducted as a split-plot experiment within a RCBD. The greenhouse experiment was a factorial experiment based on a completely randomized design (CRD). Both experiments included three biological replicates. The irrigation regime and population were treated as fixed effects, and the block (field) as a random effect. Before ANOVA analysis, the data were first checked for normality and homogeneity of variances using the Shapiro–Wilk and Levene’s tests, respectively. Statistical analyses were performed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA), which was used to evaluate the main effects and interactions among the irrigation regimes and thyme populations. When significant differences were detected by ANOVA, Means were compared using the least significant difference (LSD) post hoc test at $p \leq 0.05$. Pearson correlation analysis was applied to assess relationships among traits. The graphs were drawn using Statgraphics ver. 16, JMP ver. 11, and the correlation heatmap and binary plot were created with online software in September 2026 (<https://www.chiplot.online>).

5. Conclusions

Drought stress induced significant morphological, physiological, biochemical, and phytochemical changes in the evaluated *Thymus* populations, with the magnitude and direction of these responses varying among populations and between experimental environments. Among the populations evaluated, *T. kotschyanus*-2 showed relatively better drought performance, characterized by comparatively smaller reductions in biomass, better maintenance of water status, favorable biochemical responses, and relatively favorable drought tolerance indices. In contrast, *T. serpyllum* showed comparatively greater sensitivity across several measured traits. These classifications represent relative performance under the experimental conditions evaluated and should not be interpreted as formal estimates of cross-environment stability because no combined population $\times$ environment model was applied. The findings identify *T. kotschyanus*-2 as a promising population for further evaluation under water-limited cultivation conditions, particularly in arid and semi-arid environments. Future studies should evaluate these populations across multiple years and locations using formal population $\times$ environment analytical frameworks to better characterize the stability and reproducibility of their drought responses. Detailed essential oil compositional analyses, such as GC–MS, together with molecular approaches, would also help elucidate the mechanisms underlying population-specific drought responses and metabolite accumulation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/stresses6040078/s1>. Table S1: Gene bank codes, accession codes, and origins of ten thyme populations. Table S2: Analysis of variance of the data for the morphological traits found for ten thyme populations after the application of different irrigation regimes under field conditions. Table S3: Analysis of variance of the data for the physiological traits found for ten thyme populations after the application of different irrigation regimes under field conditions. Table S4: Mean comparisons of photosynthetic pigments of ten thyme populations after the application of different

irrigation regimes under field conditions. Table S5: Mean comparisons of physiological traits of ten thyme populations after the application of different irrigation regimes under field conditions. Table S6: Analysis of variance of the data for the biochemical traits found for ten thyme populations after the application of different irrigation regimes under field conditions. Table S7: Mean comparisons of biochemical traits of ten thyme populations after the application of different irrigation regimes under field conditions. Table S8: Analysis of variance of the data for drought resistance indices found for ten thyme populations after the application of different irrigation regimes under field conditions. Table S9: Mean comparisons between drought tolerance indicators for ten thyme populations after the application of different irrigation regimes under field conditions. Table S10: Analysis of variance of the data for the morphological traits found for ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Table S11: Analysis of variance of the data for the physiological traits found for ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Table S12: Mean comparisons of photosynthetic pigments of ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Table S13: Mean comparisons of physiological traits of ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Table S14: Analysis of variance of the data for the biochemical traits found for ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Table S15: Mean comparisons of biochemical traits of ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Table S16: Analysis of variance of the data for drought resistance indices found for ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Table S17: Mean comparisons between drought tolerance indicators in ten thyme populations after the application of different irrigation regimes under greenhouse conditions. Figure S1: Calibration curves of polyphenolic standards. Figure S2: Pearson correlation coefficients show a significant positive correlation between most drought tolerance indicators and shoot dry weight in ten thyme populations (see Table S1) after the application of different irrigation regimes under field conditions. Figure S3: Pearson correlation coefficients show a significant positive correlation between most drought tolerance indicators and shoot dry weight in ten thyme populations (see Table S1) after the application of different irrigation regimes under greenhouse conditions. Figure S4: HPLC chromatographic separation of methanolic extract of *Thymus*. Peaks identified. Figure S5: The major phenolic and flavonoid compounds of three selected thyme species based on HPLC analysis under field conditions. Figure S6: The major phenolic and flavonoid compounds of three selected thyme species based on HPLC analysis under greenhouse conditions.

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Abbreviations

The following abbreviations are used in this manuscript:

FC	Field Capacity
C	Control
MWS	Moderate Water Stress
SWS	Severe Water Stress
MP	Mean Productivity
STI	Stress Tolerance Index
GMP	Geometric Mean Productivity
TOL	Tolerance Index
SSI	Stress Susceptibility Index
YSI	Yield Stability Index
Ys	Shoot dry weight under stress conditions
Yp	Shoot dry weight under non-stress conditions
EO	Essential Oil
RWC	Relative Water Content
EL	Electrolyte Leakage
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
HPLC	High-Performance Liquid Chromatography

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