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Establishing stress conditions and physiological parameters for studying drought and broomrape stress responses in sunflower (*Helianthus annuus* L.)

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Summary: With the increasing frequency and intensity of droughts and the threat of new pathogen populations due to climate change, understanding plant responses to stresses is crucial. This work studies the physiological effects of drought stress in sunflowers (*Helianthus annuus* L.) to set repeatable water stress procedures which mimic field stress conditions, for investigating stress response at transcriptomic and epigenomic level. Two sunflower lines were tested: ABOR6 (drought-resistant) and DFAB1 (drought-susceptible) grown under controlled greenhouse conditions. Also, the reaction of the drought resistant sunflower line to the parasitic plant *Orobanche cumana* was assessed in an independent experiment. In the case of *O. cumana*, the reaction of the line to be assessed was compared with the one of a susceptible confectionary control by means of inoculation by sowing in parasite seed-infested soil. A drought stress treatment with recovery was applied, followed by measurement and comparison of physiological parameters, such as stomatal conductance, chlorophyll content, transpiration rate, nitrogen balance index. Our results showed that the chosen physiological parameters can be used to monitor the sunflower plants response to drought stress and determine the time necessary for a complete recovery from it. The findings can contribute to a deeper understanding of sunflower physiology under water stress and potentially guide the development and breeding of drought-resistant sunflower cultivars in the face of a changing climate.
Keywords: broomrape, *Heliathus annuus*, O*robanche cumana*, plant stress response, stress recovery, stomata closure, sunflower, water stress

Introduction

Sunflower (*Helianthus annuus* L.) is a diploid species (2n = 34) belonging to the family Compositae and the genus Helianthus, with a base chromosome number of 17. Its genome is relatively large, estimated at approximately 3.5 Gb (Baack et al., 2005). This considerable genome size, along with a high proportion of repetitive elements—accounting for up to 78% of the total DNA—has historically posed challenges for genomic studies (Kane et al., 2011). Morphologically, sunflower is well adapted to its ecological niche. It develops a deep, exploratory taproot system that provides structural support and enhances water uptake, particularly during dry periods. The stem is typically unbranched, thick, and covered with fine

pubescence, enabling the plant to reach considerable height. Leaves are broad, alternately arranged, and possess serrated margins, which optimize light interception and facilitate efficient photosynthesis. Although sunflower exhibits moderate drought tolerance at maturity, adequate and consistent moisture is crucial during the early stages of development. The inflorescence is a composite structure composed of sterile ray florets and fertile disc florets. The disc florets contain both male and female reproductive organs and, upon successful pollination, develop into achenes—single-seeded fruits commonly referred to as sunflower seeds.

Native to North and Central America, sunflower is now widely cultivated worldwide, primarily for its seeds, which serve both food and oil production purposes. Commercial cultivation is generally divided into two categories: one for edible seeds and the other—comprising the majority—for oil extraction (Zoumpoulakis et al., 2017). Sunflower oil is among the most widely consumed vegetable oils globally, appreciated for its favorable fatty acid composition and nutritional profile (Baydar & Erbas, 2005). Sunflower seeds are a valuable nutritional source, rich in antioxidants, flavonoids, polyunsaturated fatty acids, phenolic compounds, and essential vitamins. They also provide key micronutrients such as selenium, magnesium, copper, phosphorus, and B-complex vitamins—including thiamine, niacin, and folate—as well as vitamin E, a potent antioxidant (Guo et al., 2017; Malunjkar et al., 2024).

However, climate change has significantly impacted sunflower cultivation, especially in Southern Europe. This region is increasingly affected by rising temperatures, reduced precipitation, greater interannual variability, and more frequent extreme weather events. These climatic shifts result in shortened growing seasons, heightened water deficits, and increased heat stress, ultimately leading to yield reduction, greater yield variability, and a contraction of suitable agricultural areas (Moriondo & Bindi, 2007). Drought is a major abiotic stress limiting sunflower growth and yield, especially in arid and semi-arid regions. With agriculture using ~70% of global freshwater, increasing water scarcity worsens drought's impact (Boretti & Rosa, 2019). Water deficit in sunflower reduces seed and oil yield by impairing leaf expansion and transpiration regulation (García-López et al., 2014). At the molecular level, drought downregulates photosynthetic proteins while upregulating those involved in energy metabolism and stress responses (Dinakar et al., 2012), and contributes to oxidative stress and photosynthetic inhibition (Chaves et al., 2008).

Broomrape (*Orobanche cumana* Wallr.), a root parasite that only infects sunflower, is a major biotic constraint, particularly in Europe and Asia. Genetic resistance remains the most effective control method against its increasingly virulent races A–G (Molinero-Ruiz et al., 2008). It causes over €2 billion in global annual seed losses (Cvejic et al., 2020). Both abiotic and biotic stresses alter sunflower's phenotypic, physiological, and biochemical traits. Reported effects include reduced plant height, stem diameter, leaf number, relative water content (RWC), photosynthetic activity, increased root length and root-to-shoot ratio, stomatal closure, membrane instability, water potential decline, and ROS imbalance (Sadras et al., 1993; Hoekstra et al., 2001; Soleimanzadeh, 2012). This study investigates the physiological impacts of drought in sunflower and evaluates the drought-resistant line's response to *O. cumana*. Findings will support the identification of genes and markers linked to stress tolerance and aid in developing more resilient sunflower varieties.

Materials and methods

All the experiments were conducted in greenhouse facilities in Spain (Alameda del Obispo campus in Cordoba, from September to December 2023) and Italy (Agripolis Campus of the University of Padua in the Veneto region, during the months of April and May 2024).

Drought stress treatments

For determining the optimal substrate for plant growth and stress treatments, 60 seeds each of ABOR6 (drought-resistant) and DFAB1 (drought-susceptible) lines were sterilized and sown in small vases (diameter: 7 cm, height: 6 cm and volume: 150 ml) in 3 different substrates with varying ratios of peat and sand on 19th March 2024. The first substrate was 100% peat, and the other two substrates were a mixture of peat and river sand in the ratio of 2:1 and 1:1 respectively. They were watered regularly until

germination. Post-germination on 3rd April, the seedlings with two true leaves were moved to larger pots (diameter: 18 cm, height: 17 cm and volume: 3500 ml) into the same three substrates. Plants from each group were divided into two sub-groups: control and stress, where the latter group was subjected to water stress treatment. All the plants were watered adequately every day till when treatment began with plants having 5th and 6th leaves. During the treatment on 15th April, the plants in the control group (T0) received 400 ml water while the stress group received only 200 ml of water. They were watered with the same volume on the 19th and 23rd April. The leaf parameters were measured before watering with DUALEX® Optical Leaf Clip Meter and LI-600 Porometer on the 15th and 23rd of April from the youngest mature leaves (5th and 6th) to keep an eye on the growth and state of plants. On 24th April (T1), the stress treatment was finished. So, the leaf parameters were again measured with DUALEX® and LI-600 from the youngest mature leaves (7th and 8th) to monitor plant stress response. At the end of the treatments (T1), leaf parameters were measured and then the watering of plants in both the control and stress groups was equalized (and increased gradually from 400 ml to 700 ml) to make the drought-stressed plants recover to the pre-stress physiological state. On 3rd May (T2), the recovery treatment was completed and once again the leaf parameters were measured with DUALEX® and LI-600 from the youngest mature leaves (9th and 10th) to monitor the growth and state of plants after the water stress. For determining the optimal drought stress protocol, the growth substrate mixture of peat and river sand in the ratio of 2:1 was chosen. One hundred seeds of ABOR6 and DFAB1 lines were sterilized and sown in small pots (diameter: 7 cm, height: 6 cm and volume: 150 ml) on 5th April 2024. They were watered regularly until germination. Post-germination on 16th April, the seedlings with two true leaves were moved to larger pots (diameter: 14 cm, height: 12 cm and volume: 1100 ml, on the same substrate. All the plants from both varieties were equally divided into 3 technical replicates placed at different positions on the platform together, namely Replica 1, Replica 2 and Replica 3. The plants in each technical replicate were divided into two sub-groups: control and stress, where the latter group was induced with water stress treatment. All the plants were watered adequately with 100 ml every day but this time the drought treatment began when the plants had just started developing 3rd and 4th leaf. On 19th April (T0), the stress treatment was started and plants in the control group received 300 ml while the ones in the stress group received only 150 ml of water. The leaf parameters were measured with DUALEX® and LI-600 on the same day from the youngest mature leaves (1st and 2nd). They were watered with the same volume on the 25th and 29th of April. On 1st May (T1), the stress treatment was terminated, and the leaf parameters were again measured with DUALEX® and LI-600 from the youngest mature leaves (3rd and 4th). At this point, the watering of plants in both control and stress groups was equalised to 300 ml to make the drought-stressed plants recover. On 8th May (T2), the recovery period ended, and leaf parameters were again measured with DUALEX® and LI-600 from the youngest mature leaves (5th and 6th).

Growth parameter measurements on leaf under drought stress.

Plant growth parameters were measured using the DUALEX® Optical Leaf Clip Meter and the LI-600 Porometer. The DUALEX® device assesses chlorophyll, flavonols, anthocyanins, and Nitrogen Balance Index (NBI), which reflects nitrogen availability via the chlorophyll-to-flavonoid ratio—higher NBI values indicate better nitrogen status (de Souza et al., 2022). Chlorophyll is measured through transmittance at far-red and near-infrared wavelengths, while flavonols and anthocyanins are detected by comparing chlorophyll fluorescence under specific light wavelengths. This approach, known as the screening effect, quantifies how polyphenols affect light reaching the chlorophyll (DUALEX® Manual, 2019).

The LI-600 combines a porometer and a Pulse-Amplitude Modulation (PAM) fluorometer to measure stomatal conductance and chlorophyll-a fluorescence. Stomatal conductance to water vapour (gsw), which reflects the degree of stomatal opening, is influenced by environmental factors like light, CO_2, and humidity. The device uses a flow-through system to measure transpiration (E) and calculates gsw from the total conductance (gtw) and boundary layer conductance (gbw), providing insight into water loss regulation and gas exchange.

Statistical analysis of responses to drought stress

Brown-Forsythe and Welch Test in one-way Analysis of Variance (ANOVA) were performed to compare the results of plants from control and water-stressed groups using a P-value threshold of 0.05, with P-values below 0.001 being considered highly significant. To find the correlation between Transpiration Rate and Vapour Pressure Deficit, Pearson correlation coefficient (PCC) was calculated using a confidence interval of 95%. The graphs and statistical analysis of the data were then made using GraphPad Prism (v8.0.2).

Assessment of reaction to Orobanche cumana

The *O. cumana* population Oc0115, characterized as race F (Gómez-Lama Cabanás et al., submitted), was used to inoculate the sunflower confectionary cultivar B117 (susceptible to all parasite races) (Molinero-Ruiz et al., 2008) and inbred line ABOR6 (of unknown reaction to *O. cumana*). The experiment had a completely randomised factorial design, genotype and parasite being the two factors. Seed of *O. cumana* was used to inoculate 6 seedlings (replications) of each of the sunflower genotypes. Sunflower seeds were surface-sterilised by immersing them in 10% sodium hypochlorite for 5–10 min, then thoroughly rinsed in deionised water and incubated in the dark at saturation humidity in a germinator at 24–28 °C until radicles were 2–5 mm long. Each sunflower line was inoculated by transplant of individual seedlings to pots with 400 ml of soil mixture SS (sand:silt, 1:1, V / V) uniformly infested with Oc0115 seed (0.103 mg seeds per g SS). Six control seedlings of each genotype were individually transplanted in pots of the same size containing uninfested SS. After 14 days of growth under optimal conditions for infection in greenhouse (20–25 °C and photoperiod of 14 h day-1), sunflower plantlets were transplanted, with the soil, to 2 litre pots containing soil mixture sand:silt:peat moss (2:1:3, V / V) and grown in the greenhouse under the same conditions. The degree of attack (DA, number of emerged *O. cumana* stems per plant) was assessed weekly until senescence of the sunflower. At the end of the experiment, records on broomrape incidence (BI, expressed as the percentage of sunflower plants with emerged parasite stems), on final DA, and on number of broomrape nodules per sunflower plants were taken. Height of plants and dry weights of roots and aboveground organs of sunflower, and dry weight of emerged *O. cumana* stems were also recorded. For determination of dry weights, plant tissues were kept in stove at 80 ºC for 48 h.

Results and Discussion

Upon comparing the final growth status of the plants from the tree tested substrates, it was evident that the plants grown in the substrate mix containing peat and river sand in the ratio of 2:1 had the best growth and morphology. They were taller than the plants grown in the other two substrates, along with having bigger and broader leaves (Figure 1). Additionally, we observed this mixture also had the best water percolation among the 3 contender substrates, preventing excessive water retention.

On the selected substrate, water stress was applied when the plants started to grow their 3rd and 4th leaves, to evaluate the two lines responses at earlier phases of development. Water-stressed plants start to display drought-affected traits and morphology such as retarded growth and yellowish wilted leaves within a week of reduced watering. They keep growing despite the water deficiency, albeit at a much lower rate compared to the plants of the control. At the end of the drought stress induction period (T1), there was a significant difference in the growth and overall morphology between control and stressed plants. Plants from the control were taller and had broader leaves than the water-stressed plants both in the DFAB1 (drought-susceptible) and ABOR6 (drought-resistant) genic lines (Figures 2 and 3).

The physiological data measured with LI-600 and DUALEX® are presented in Tables 1 and 2 and support the visual observations of stress-affected plants subjected to the drought stress protocol. Graphs are displayed for relevant parameters showing significant differences between plants of water-stressed and control groups. Average, standard deviation and statistical analysis were calculated considering all the plants from the three replicates as a single population.

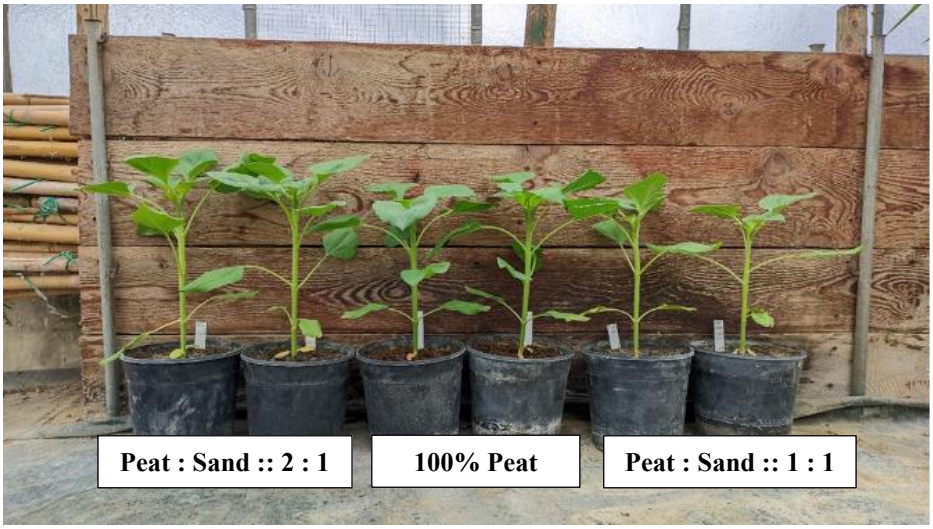

Figure 1. Growth comparison of sunflower plants in the three substrates

Figure 2. Growth comparison of DFAB1 plants at the end of T1
(left: control plants, right: water-stressed plants

Figure 3. Growth comparison of ABOR6 plants at the end of T1
(left: control plants, right: water-stressed plants)

Table 1. Physiological data of plants at the end of drought stress treatment from both varieties measured with LI-600 (T1)

Line	gsw (Stomatal Conductance) mol/m2*s	gtw (Total Conductance) mol/m2*s	E_Apparent (Transpiration Rate) mmol/m2*s	VPleaf (Vapour pressure) Kpa	PhiPS2 (Quantum Light Efficiency)
DFAB1 Control	0.721 ± 0.03	0.576 ± 0.02	10.384 ± 1.38	3.69 ± 0.29	0.74 ± 0.03
DFAB1 Stressed	0.205 ± 0.054	0.187 ± 0.045	4.894 ± 1.472	4.488 ± 0.301	0.733 ± 0.026
ABOR6 Control	0.87 ± 0.056	0.666 ± 0.034	11.62 ± 1.69	3.71 ± 0.188	0.7 ± 0.024
ABOR6 Stressed	0.332 ± 0.131	0.289 ± 0.104	7.016 ± 1.738	4.426 ± 0.47	0.704 ± 0.018

Table 2. Physiological data of plants at the end of drought stress treatment from both varieties measured with DUALEX® (T1)

Line	Chlorophyll Index	Anthocyanin Index	Flavanol Index	Nitrogen Balance Index
DFAB1 Control	24.26811±1.64202	0.19067±0.00577	0.77833±0.02634	31.79756±4.45538
DFAB1 Stressed	27.03233±2.12248	0.18327±0.00274	0.58764±0.10101	48.4705±10.73797
ABOR6 Control	23.1445±1.19254	0.1985±0.00425	0.83889±0.04368	27.93717±2.93102
ABOR6 Stressed	26.14517±0.9705	0.18042±0.00136	0.69409±0.04595	38.49167±4.22297

Physiological data at the end of drought stress treatment (T1, Figure 4 and 5) indicated that the water-stressed plants of the DFAB1 genic line had highly significant lower values (P-value < 0.001) of Stomatal Conductance (gsw) as well as Transpiration Rate (E_apparent) compared to the control group. Similarly, water-stressed plants from the ABOR6 line presented highly significant lower values (P-value < 0.001) of Stomatal Conductance as well as Transpiration Rate.

Concerning the Chlorophyll index and Nitrogen Balance Index (NBI), water-stressed plants of the DFAB1 genic line showed highly significant greater values compared to the control plants (P-value < 0.001). Similarly, stressed plants from the ABOR6 line showed highly significant higher values (P-value < 0.001) of chlorophyll content as well as the Nitrogen Balance Index.

After the drought stress period, during the recovery phase water-stressed plants returned to pre-stressed morphological and physiological condition almost after a week of increased watering. They looked comparable to their control in terms of morphology (Figure 6 and 7).

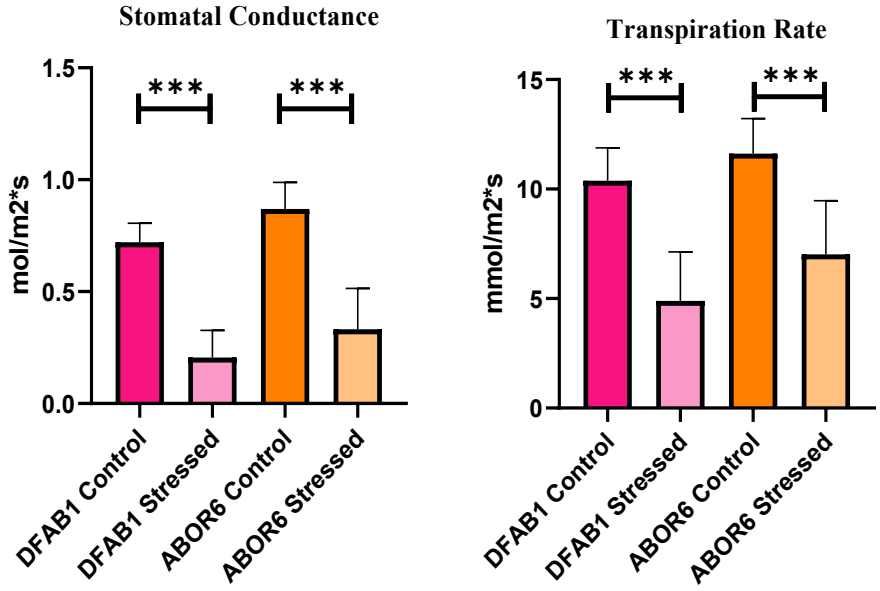

Figure 4. Stomatal Conductance and Transpiration Rates of plants from Control and Water-stressed group for both genic lines at T1 (***=Highly Significant, P-value < 0.001)

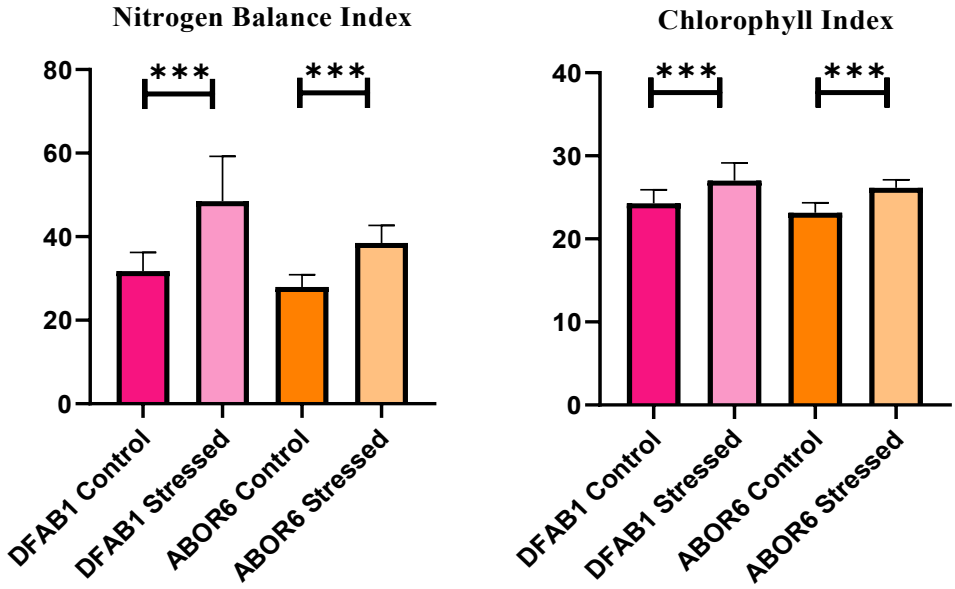

Figure 5. Chlorophyll Index and Nitrogen Balance Index of plants from control and water-stressed group for both genic lines at T1 (***= Highly Significant, P-value < 0.001)

Figure 6. DFAB1 plants at the end of T2 (left: control, right: water stressed)

Figure 7. ABOR6 plants at the end of T2 (left: control, right: water stressed)

Physiological data measured from LI-600 and DUALEX® and the respective relevant graphs after the end of recovery period (T2) are showed in Tables 3 and 4 and Figures 8 and 9. Plants of both genic lines from both the control and water-stressed groups had remarkably similar values of stomatal conductance and transpiration rate (P-value > 0.05). For the chlorophyll index, stressed plants from the DFAB1 genic line showed highly significant lower values than the control group. The same trend is observed for ABOR6, even if the difference here is not significant. DFAB1 stressed plants show a very similar average value of nitrogen balance index with control plants, while ABOR6 stressed plants show a significant higher value (P-value < 0.05). Overall, we concluded that at the end of the recovery period, the physiological parameters of the stressed plants were mostly comparable to the non-stressed plants.

Table 3. Physiological data of plants at the end of recovery period from both varieties measured with LI-600 (T2)

Line	gsw (Stomatal Conductance) mol/m2*s	gtw (Total Conductance) mol/m2*s	E_Apparent (Transpiration Rate) mmol/m2*s	VPleaf (Vapour pressure) Kpa	PhiPS2 (Quantum Light Efficiency)
DFAB1 Control	0.365 ± 0.052	0.322 ± 0.042	5.296 ± 0.836	2.715 ± 0.311	0.692 ± 0.023
DFAB1 Stressed	0.376 ± 0.026	0.332 ± 0.02	5.391 ± 1.039	2.677 ± 0.227	0.701 ± 0.004
ABOR6 Control	0.328 ± 0.021	0.293 ± 0.017	4.831 ± 0.902	2.662 ± 0.236	0.705 ± 0.006
ABOR6 Stressed	0.37 ± 0.074	0.326 ± 0.058	5.165 ± 1.357	2.585 ± 0.145	0.674 ± 0.056

Table 4. Physiological data of plants at the end of recovery period from both varieties measured with DUALEX® (T2)

Line	Chlorophyll Index	Anthocyanin Index	Flavanol Index	Nitrogen Balance Index
DFAB1 Control	27.61483±0.72253	0.16674±0.00332	0.66105±0.04472	42.94125±3.11566
DFAB1 Stressed	24.47583±1.469	0.16412±0.00338	0.59262±0.06931	43.36817±7.93463
ABOR6 Control	21.14017±1.91047	0.19392±0.00298	0.73525±0.03924	29.59542±4.58687
ABOR6 Stressed	18.65408±4.00278	0.22512±0.00716	0.51195±0.0279	38.8565±9.80973

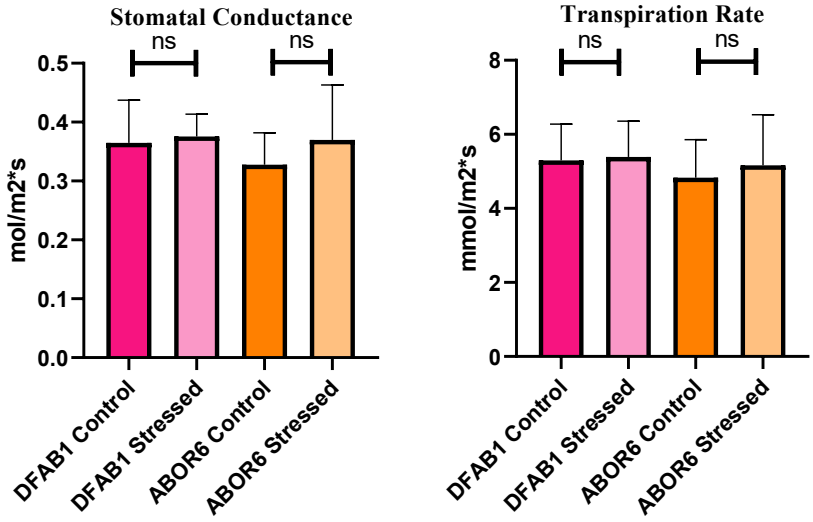

Figure 8. Stomatal conductance and transpiration rates of plants from control and water-stressed group for both genic lines at T2 (ns=No Significant Difference)

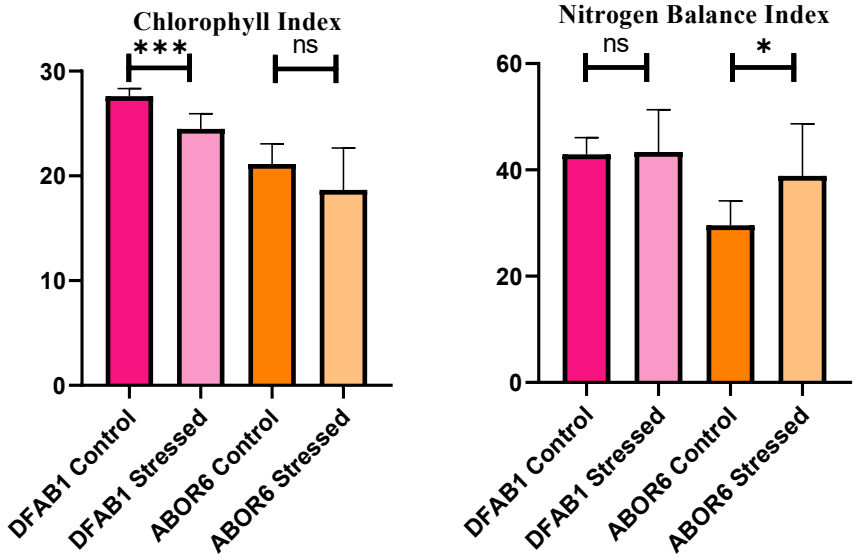

Figure 9. Chlorophyll Index and Nitrogen Balance Index of plants from control and water-stressed group for both genic lines at T2 (*= Significant, ***= High Significance, ns=No Significant Difference)

Water stress induces stomatal closure, reducing CO_2 uptake and photosynthesis, which inhibits plant growth (Zargar et al., 2017). However, after rewatering, growth differences between control and stressed plants were minimal, suggesting a capacity for recovery through restored photosynthetic activity. Stomatal conductance—the rate of gas exchange through leaf stomata—is influenced by stomatal aperture and regulated by environmental factors such as light, temperature, humidity, and CO_2 concentration (Monteith & Unsworth, 2013). Lower stomatal conductance and transpiration rates in stressed plants indicate a water-conservation strategy during drought (Onyemaobi et al., 2021). Drought also increases vapour pressure deficit (VPD), raising evaporative demand and affecting leaf gas exchange. Under high VPD, plants with low hydraulic conductance limit transpiration earlier (Sancho-Knapik et al., 2022; Koehler et al., 2023).

While drought often reduces chlorophyll content, drought-tolerant species like sunflower may instead increase chlorophyll and Nitrogen Balance Index as adaptive responses. Elevated chlorophyll could enhance light absorption and photosynthetic efficiency, allowing plants to better utilize nitrogen and maintain metabolic function despite restricted CO_2 uptake (Ashraf & Siddiqi, 2024).

Concerning the reaction to *O. cumana*, initial broomrape stems in the susceptible control B117 emerged from the soil six weeks after inoculation, its number progressively increasing along the following weeks (Table 5). Twelve weeks after inoculation, visible broomrape stems did not increase further, and the experiment was finalised. Significant differences of BI, final DA, stems' dry weight and number of nodules were obtained for genotype and parasite ($p<0.001$ in both cases) but not for their interaction. Broomrape incidences were 0 or 100% and corresponded to fully resistant or susceptible reactions, respectively, of the sunflower genotypes.

Table 5. Reaction of the susceptible cv. B117 and the inbred line ABOR6 to the inoculation with *Orobanche cumana* race F after incubation under greenhouse conditions

				Dry weight of aboveground organs (g)	Broomrape infection			
Genotype	Treatment	Height (cm)	Dry weight of roots (g)		BI (%)	Final DA (stems per plant)	Dry weight of stems (g)	Nodules (N)
B117	Control	95.8	1.78	4.23	0	0	0	0
	Inoculated	86.9	0.64	3.31	100	4.67	1.59	4.83
ABOR6	Control	92.1	1.69	3.42	0	0	0	0
	Inoculated	89.2	1.58	2.97	0	0	0	0

Genotype B117 was susceptible (BI 100%) and had final DA of 4.67 broomrape stems per sunflower plant (Figure 10A) and 4.83 broomrape nodules per sunflower plants (Figure 10B). Dry weights of broomrape stems were 1.59 g. No differences of height and dry weights of aboveground organs and roots were observed neither in inoculated B117 nor in inoculated ABOR6 as compared with the control plants. Inbred line ABOR6 was resistant to population Oc0115: BI and final DA were 0% and 0 broomrape stems per sunflower plant, respectively (Figure 10C). Interestingly, our results show that sunflower material from IFVCNS includes at least one line with resistance to virulent races of broomrape, and that this line can be used in breeding for both drought tolerance and resistance to *O. cumana*.

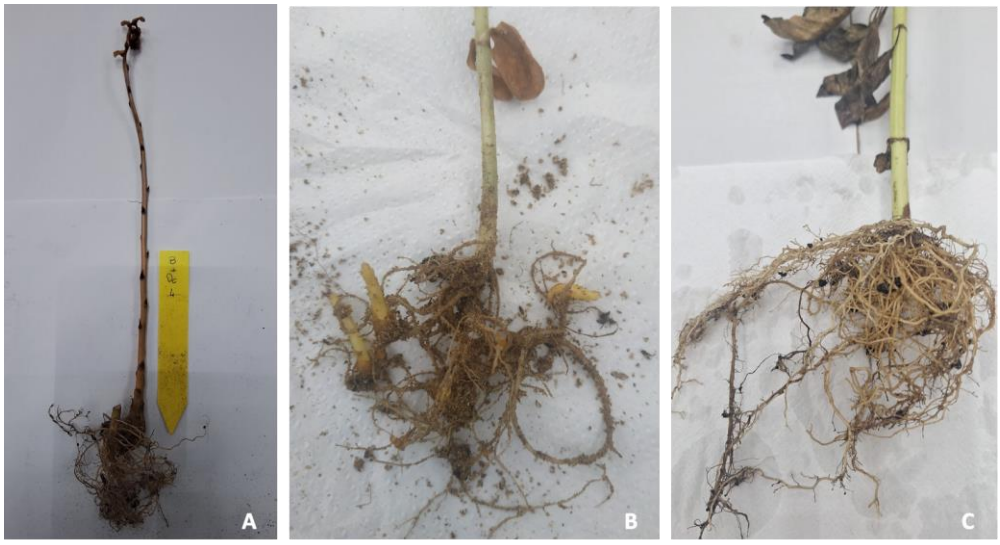

Figure 10. Infection by *O. cumana* in the susceptible confectionary sunflower cultivar B117: broomrape stem (A, the sunflower stem was cut) and nodules (B), and healthy roots of the sunflower inbred line ABOR6 (C)

Previous works by our research group suggest that broomrape-infected sunflowers susceptible to *O. cumana* have, among others, higher leaf temperature (Ortiz-Bustos et al., 2016) and lower stomatal conductance (García-Carneros et al., 2025). In this work, these same alterations were observed in plants under drought stress. Future studies should investigate whether the physiological alterations observed in response to *O. cumana* infection and drought stress share common or distinct genetic regulatory mechanisms in sunflower genotypes susceptible to both stresses. Additionally, exploring whether genetic resistance to *O. cumana* and drought tolerance are controlled by overlapping or independent genetic pathways could represent a valuable direction for future research.

Author contributions: Alessia Ronchi, Shaurya Kumar Lal, Irene Luzzi e Anna Maria Placentino performed the drought stress treatments in the greenhouse and collected the data on plants. Alessia Ronchi and Shaurya Kumar Lal did the statistical analyses on collected data. Leire Molinero-Ruiz and Ana Belen Garcia-Carneros performed the experiments and data analysis on *Orobanche cumana*. Serena Varotto coordinated the work assembly and writing. All authors contributed to the writing of the manuscript.

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References

Ashraf, F., Siddiqi, E. (2024). Mitigation of drought-induced stress in sunflower (Helianthus annuus L.) via foliar application of Jasmonic acid through the augmentation of growth, physiological, and biochemical attributes. *BMC Plant Biology*, 24, 592. https://doi.org/10.1186/s12870-024-05273-4

Baack, E. J., Whitney, K. D., & Rieseberg, L. H. (2005). Hybridization and genome size evolution: timing and magnitude of nuclear DNA content increases in Helianthus homoploid hybrid species. *New Phytologist*, *167*(2), 623–630. https://doi.org/10.1111/j.1469-8137.2005.01433.x

Baydar, H., & Erbas, S. (2005). Influence of seed development and seed position on oil, fatty acids and total tocopherol contents in sunflower (Helianthus annuus L.). *Turkish Journal of Agriculture and Forestry*, *29*(3), 179–186.

Boretti, A., & Rosa, L. (2019). Reassessing the projections of the World Water Development Report. *Npj Clean Water*, *2*(1). https://doi.org/10.1038/s41545-019-0039-9

Buriro, M., Sanjrani, A., Chachar, Q., Ahmed, N., Chachar, S., Buriro, B., Gandahi, A. W., & Mangan, T. (2015). Effect of water stress on growth and yield of sunflower. *Journal of Agricultural Technology*, *11*(7), 1547–1563.

Chaves, M. M., Flexas, J., & Pinheiro, C. (2008). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, *103*(4), 551–560. https://doi.org/10.1093/aob/mcn125

Cvejić, S., Radanović, A., Dedić, B., Jocković, M., Jocić, S., & Miladinović, D. (2020). Genetic and Genomic Tools in Sunflower Breeding for Broomrape Resistance. *Genes*, *11*(2), 152. https://doi.org/10.3390/genes11020152

de Souza, R., Peña-Fleitas, M. T., Thompson, R. B., Gallardo, M., Grasso, R., & Padilla, F. M. (2022). Use of fluorescence indices as predictors of crop N status and yield for greenhouse sweet pepper crops. *Precision Agriculture*, *23*(1), 278–299. https://doi.org/10.1007/s11119-021-09837-4

Dinakar, C., Djilianov, D., & Bartels, D. (2012). Photosynthesis in desiccation tolerant plants: Energy metabolism and antioxidative stress defense. *Plant Science*, *182*, 29–41. https://doi.org/10.1016/j.plantsci.2011.01.018

García-Carneros et al. 2025. Reaction of sunflower material from IFVCNS to *O. cumana* race F and a sensor-based initial approach for early detection of infections. P. 11 in *Book of Abstracts CROPINNO Final Conference "New Approaches in Crop Improvement for Increased Climate Resilience"*, 26 June 2025, Novi Sad, Serbia.

García-López, J., Lorite, I. J., García-Ruiz, R., & Domínguez, J. (2014). Evaluation of three simulation approaches for assessing yield of rainfed sunflower in a Mediterranean environment for climate change impact modelling. *Climatic Change*, *124*(1–2), 147–162. https://doi.org/10.1007/s10584-014-1067-6

Ghobadi, M., Taherabadi, S., Ghobadi, M.-E., Mohammadi, G.-R., & Jalali-Honarmand, S. (2013). Antioxidant capacity, photosynthetic characteristics and water relations of sunflower (Helianthus annuus L.) cultivars in response to drought stress. *Industrial Crops and Products*, *50*, 29–38. https://doi.org/10.1016/j.indcrop.2013.07.009

Gómez-Lama Cabanás et al. (submitted). Exploring the biocontrol of *Orobanche cumana* (sunflower broomrape) race F and the promotion of sunflower growth by rhizospheric bacterial strains indigenous to Spain.

Guo, S., Ge, Y., & Na Jom, K. (2017). A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (Helianthus annuus L.). *Chemistry Central Journal*, *11*(1), 95. https://doi.org/10.1186/s13065-017-0328-7

Hoekstra, F. A., Golovina, E. A., & Buitink, J. (2001). Mechanisms of plant desiccation tolerance. *Trends in Plant Science*, *6*(9), 431–438. https://doi.org/10.1016/S1360-1385(01)02052-0

Hussain, S., Saleem, M. F., Ashraf, M. Y., Cheema, M. A., & Haq, M. A. (2010). Abscisic acid, a stress hormone helps in improving water relations and yield of sunflower (Helianthus annuus L.) hybrids under drought. *Pakistan Journal of Botany*, *42*(3), 2177–2189.

Kane, N. C., Gill, N., King, M. G., Bowers, J. E., Berges, H., Gouzy, J., Bachlava, E., Langlade, N. B., Lai, Z., Stewart, M., Burke, J. M., Vincourt, P., Knapp, S. J., & Rieseberg, L. H. (2011). Progress towards a reference genome for sunflower. *Botany*, *89*(7), 429–437. https://doi.org/10.1139/b11032

Koehler, T., Wankmuller, F. J. P., Sadok, W., & Carminati, A. (2023). Transpiration response to soil drying versus increasing vapor pressure deficit in crops: physical and physiological mechanisms and key plant traits. *Journal of Experimental Botany*, 74(16), 4789–4807. https://doi.org/10.1093/jxb/erad221

Kramer, P. J., & Boyer, J. S. (1995). *Water relations of plants and soils*. Academic Press.

Malunjkar, B., Lokhande, R., & Chitodkar, S. (2024). The significance of sunflower in ecology and agriculture. *5*, 811–813.

Molinero-Ruiz et al. 2008. Indigenous highly virulent accessions of the sunflower root parasitic weed *Orobanche cumana*. *Weed Research*, 48: 169-178. https://doi.org/10.1111/j.1365-3180.2007.00611.x

Moriondo, M., & Bindi, M. (2007). Impact of climate change on the phenology of typical mediterranean crops. *Italian Journal of Agrometeorology*, *3*, 5–12.

Monteith, J., & Unsworth, M. (2013). *Principles of environmental physics: plants, animals, and the atmosphere*. Academic press.

Onyemaobi, O., Sangma, H., Garg, G., Wallace, X., Kleven, S., Suwanchaikasem, P., Roessner, U., & Dolferus, R. (2021). Reproductive Stage Drought Tolerance in Wheat: Importance of Stomatal Conductance and Plant Growth Regulators. *Genes*, 12(11), 1742. https://doi.org/10.3390/genes12111742

Ortiz-Bustos et al. (2017). Use of blue-green fluorescence and thermal imaging in the early detection of sunflower infection by the root parasitic weed *Orobanche cumana* Wallr. *Frontiers in Plant Science*, 8, 833. https://doi.org/10.3389/fpls.2017.00833

Sadras, V. O., Villalobos, F. J., & Fereres, E. (1993). Leaf expansion in field-grown sunflower in response to soil and leaf water status. *Agronomy Journal*, 85(3), 564–570. https://doi.org/10.2134/agronj1993.00021962008500030009x

Sancho-Knapik, D., Mendoza-Herrer, Ó., Alonso-Forn, D., Saz, M. Á., Martín-Sánchez, R., dos Santos Silva, J. V., Ogee, J., Peguero-Pina, J. J., Gil-Pelegrín, E., & Ferrio, J. P. (2022). Vapor pressure deficit constrains transpiration and photosynthesis in holm oak: A comparison of three methods during summer drought. *Agricultural and Forest Meteorology*, 327, 109218. https://doi.org/10.1016/j.agrformet.2022.109218

Soleimanzadeh, H. (2012). Response of Sunflower (Helianthus annuus L.) To selenium application under water stress. *World Applied Sciences Journal*, *17*(9), 1115–1119. http://www.idosi.org/wasj/wasj17(9)12/7.pdf

Zargar, S. M., Gupta, N., Nazir, M., Mahajan, R., Malik, F. A., Sofi, N. R., Shikari, A. B., & Salgotra, R. K. (2017). Impact

of drought on photosynthesis: molecular perspective. *Plant Gene*, *11*, 154–159. https://doi.org/10.1016/j.plgene.2017.04.003

Zoumpoulakis, P., Sinanoglou, V., Siapi, E., Heropoulos, G., & Proestos, C. (2017). Evaluating modern techniques for the extraction and characterisation of sunflower (Hellianthus annus L.) Seeds Phenolics. *Antioxidants*, *6*(3), 46. https://doi.org/10.3390/antiox6030046

Određivanje stresnih uslova i fizioloških parametara za proučavanje odgovora suncokreta (*Helianthus annuus* L.) na stres izazvan sušom i volovodom

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Sažetak: Sa pojačanom učestalošću i intenzitetom suša i pretnjom novih populacija patogena usled klimatskih promena, razumevanje odgovora biljaka na stres je ključno. Ovaj rad proučava fiziološke efekte stresa suše kod suncokreta (*Helianthus annuus* L.) kako bi se ustanovile ponovljive procedure vodnog stresa koje imitiraju uslove stresa na polju, za ispitivanje odgovora na stres na transkriptomskom i epigenomskom nivou. Testirane su dve linije suncokreta: ABOR6 (otporan na sušu) i DFAB1 (osetljiv na sušu) gajene u kontrolisanim uslovima staklene bašte. Takođe, reakcija linije suncokreta otporne na sušu na parazitsku biljku *Orobanche cumana* procenjena je u nezavisnom eksperimentu. U slučaju *O. cumana*, reakcija linije koja se procenjuje upoređena je sa reakcijom osetljive kontrolne konzumne linije putem inokulacije setvom u zemljište zaraženo semenom parazita. Primenjen je tretman stresa izazvanog sušom sa oporavkom, nakon čega je usledilo merenje i poređenje fizioloških parametara, kao što su provodljivost stoma, sadržaj hlorofila, brzina transpiracije, indeks azotnog bilansa. Naši rezultati su pokazali da se izabrani fiziološki parametri mogu koristiti za praćenje odgovora biljaka suncokreta na stres izazvan sušom i određivanje vremena potrebnog za potpuni oporavak od njega. Nalazi mogu doprineti dubljem razumevanju fiziologije suncokreta pod vodnim stresom i potencijalno usmeriti razvoj i oplemenjivanje sorti suncokreta otpornih na sušu u uslovima klimatskih promena.

Ključne reči: *Helianthus annuus*, odgovor biljaka na stres, oporavak od stresa, *Orobanche cumana*, suncokret, vodni stres, volovod, zatvaranje stoma