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Genotype diversity as a source of tolerance to drought stress in legumes

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Abstract

Chickpea and lentils are one of the most important legumes not only as sources of food and nutrients but also for enrichment of soil as a nitrogen fixing crop. An early onset of higher temperatures and drought are affecting chickpea and lentil growth and flowering leading to reduction of yield. In search for a tolerant varieties presented study performed a large-scale screening of two legume varieties (chickpea and lentils) investigating phenotypical response to early onset of drought under heat stress. Under heat stress and two different irrigation conditions, 19 chickpea and 18 lentil accessions were examined. The evaluation focused on their growth, biomass production, and flowering rate in comparison to commercially available varieties. Six chickpea accessions showed tolerance to water stress while only two lentil accessions differed from the rest of tested accessions. Generally, lentils genotypes were less stressed by decreased water availability compared to chickpea. Large scale screening of legume accessions could be a valuable tool to identify new varieties that could show phenotypical traits more adaptable to climate related environmental stresses. To improve the reproductive efficiency in chickpeas and lentils under adverse conditions associated to climate change an extensive breeding effort should be focused on investigation of more tolerant genotypes and cultivation in crop systems.

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Keywords

*Climate change,
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Lentils*

Introduction

Crop biodiversity is one of the most important factors of human life change through the domestication of edible plants. It is also an essential resource for crop improvement, development of sustainable agriculture as a response to global climate change and the increase of human population, consumer preferences as well as their nutritional requirements (Hufford et al., 2019). Domestication and the consequent evolution under cultivation have drastically shaped the genetic structure of any crop biodiversity. Connecting genotype and phenotype can be considered as the holy grail of any studies of plant biodiversity (Hufford et al., 2019).

Legumes have been successfully bred for their high nutritional components, ability to assist fixation of nitrogen and for their other key ecosystem services. The most worldwide common legume crops are peas (*Pisum sativum* L.), broad beans (*Vicia faba* L.), chickpeas (*Cicer arietinum* L.), beans (*Phaseolus vulgaris* L.) and lentils (*Lens culinaris* Medikus) (Maqbool et al., 2010). Lentil is one of the first domesticated plants in the Middle East and one of the most important legumes in term of global food supply. Second fundamentally important crop in agriculture is chickpea, with undoubted role in the subsistence of a large portion of the population (Gaur et al., 2008).

There are various negative factors that contribute to reduction of the legume's gene pool diversity. For instance, monocropping, which are optimized for the productivity to match increasing food demand, result in a diversity and capacity loss of the plants making them more susceptible to environmental stresses (Foyer et al., 2016).

Worldwide, approximately half of the chickpeas and lentils yield is lost due to abiotic stresses, especially by temperature changes affecting all vegetative stages with highest effects during flowering phase (Kalve & Tadege, 2017; Rani et al., 2020). A large variety of phenotypic traits could be one of the ways to counteract the climate changes, where autochthonous ecotypes can provide greater fitness and stability of production under specific regional or stress conditions (Singh et al., 2021).

Main issue in chickpea cultivation under climate change, with the emphasis on drought and heat, is inherently narrow genetic base due to series of genetic bottlenecks because of natural selection and farmers selection pressures and use of variability in the breeding of modern cultivars (Singh et al., 2022a). The germplasm variability in chickpea is very limited (Kumar and Gugita, 2004). To improve current tolerance levels of chickpea it is necessary to explore variability of germ plasm available and to test if some genotypes are more tolerant compared to two varieties being currently cultivated.

Aim of this study was to evaluate the germplasm variability of autochthonous Italian genotype varieties of *Cicer arietinum* L. (23 genotypes) and *Lens culinaris* Medikus (22 genotypes) under the simultaneous occurrence of two abiotic stresses linked to climate change (low water regimes under high temperature) and identify most tolerant and resistant genotypes usable in further breeding activity. Performance of the varieties was measured according to their agronomic traits evaluating direct effect of stress on growth and yield of chickpea and lentil genotypes.

Material and methods

Greenhouse conditions

The experiment was conducted from February to June 2021 in the greenhouse of Agricultural Technical Institute (ITAGR), Firenze, Italy (43°47'02.2 N; 11°13'21.7 E). The maximum and minimum air temperatures ranged from 12°/3° C in February to 29°/16° C in June. The monthly mean greenhouse temperature was from 15°C (February) to 35°C (June).

Experimental design and growth conditions

The plant material used in this study is presented in Table 1. The samples examined in the presented study include 19 accessions and 4 cultivars of chickpea (Figure 1) and 18 accessions and 3 cultivars of lentil (Figure 2) mostly comprising out of accessions from Italy but also including accessions from USA and Mexico.

For the seeds germination a mixture of sand and peat soil (1:1) was prepared in plastic pots (20 x 20 cm, c.a. 2 kg in weight) with 16 pots for each accession. The pots were arranged in a completely randomized design. Approximately ten seeds were sown in each pot, at a depth of 3-4 cm and following a randomization of seed distribution within the pot. After germination, representative two seedlings per each pot were left and the rest of the seedling were removed to optimize the plant growth in later stages. During the first 60 days, each pot was watered to the field capacity to maintain a homogeneous growth for all planted varieties. After 60 days pots were separated into two groups: first group was watered to 100% of the evapo-transpirated (EVT) water while second group was watered to 25% of the EVT water

(water deficit conditions). Both groups of plants were exposed to heat stress starting from pre-flowering stage throughout the podding.

For all plants, supplementation with commercial fertilizer (NPK 7:7:7) was carried out once a week to enhance chickpeas development during the late growth phase and the flowering.

Agronomic traits measurements

Plant height (H) was recorded at three-time intervals (60, 75 and 90 days after sowing) during the experiment. Height of the plants was measured using graduated ruler from the soil level to the shoot tip of respective plant. At the harvest (after 120 days of cultivation) biomass parameters were recorded (shoot fresh weight - SFW, root fresh weight - RFW, shoot dry weight- SDW and root dry weight - RDW), biomass production was calculated according to the dry mass of the plants. Flowering rate was recorded for all plants including those that did not completed their cycle (no podding).

For the plant height data collected at 75 and 90 days were normalized in accordance with the height recorded at day 60 (beginning of two hydric treatments) thus reported values for plant growth represent the differences in growth in relation to start value at day 60.

All collected data were analysed using OriginPro (2021b) software (OriginLab Corporation, Northampton, MA, USA.); one way ANOVA was carried out for each agronomic parameter, using the Tukey post hoc test for means comparison at 1% and 0.1% probability level.

Table 1. Accessions tested in the study and their origin

<i>Cicer arietinum</i>				<i>Lens culinaris</i>			
Accession Name	Lab ID	Purveyor	Country	Accession	Lab ID	Purveyor	Country
Busetto Palazzuolo	BP	University of Palermo	Italy	Villalba	Vil	University of Palermo	Italy
Casteltermini	IAPA 12	University of Palermo	Italy	SFAX 5	S5	University of Palermo	Italy
Caltavuturo	C	University of Palermo	Italy	Onano	On	University of Palermo	Italy
San Biagio Platani Nero 6	SBP6	University of Palermo	Italy	Nera di Enna	NDE	University of Palermo	Italy
Alcamo 47	A47	University of Palermo	Italy	Beluga	Bel	University of Palermo	Italy
Rosso	R	University of Palermo	Italy	Campisi	Cps	University of Palermo	Italy
Montelepre	ML	University of Palermo	Italy	Di quattro grande	DQG	University of Palermo	Italy
Nero di Puglia	NDP	University of Palermo	Italy	Linosa 2	Lin	University of Palermo	Italy
Flip08 160C	Flip	International Center for Agricultural Research in the Dry Areas	Italy	Ustica	Ust	University of Palermo	Italy
W6 9484	W	International Center for Agricultural Research in the Dry Areas	Italy	Pantelleria	Pan	University of Palermo	Italy
111977	Cal-77	Mediterranean Germplasm Database	Italy	Mussomeli	Mss	University of Palermo	Italy
103245	Pug-45	Mediterranean Germplasm Database	Italy	110290	Pug-90	Mediterranean Germplasm Database	Italy
111973	Cal-73	Mediterranean Germplasm Database	Italy	103653	Pug-53	Mediterranean Germplasm Database	Italy
110220	Bas-20	Mediterranean Germplasm Database	Italy	111849	Bas-49	Mediterranean Germplasm Database	Italy

110410	Cam-10	Mediterranean Germplasm Database	Italy	Capracotta	CPC	University of Palermo	Italy
103267	Pug-67	Mediterranean Germplasm Database	Italy	Val di Nevola	VDN	University of Palermo	Italy
PI518248	USA	United State Department of Agriculture	USA	Monti Sibillini	MS	University of Palermo	Italy
PI572491	MEX	United State Department of Agriculture	Mexico	Locale	Loc	University of Palermo	Italy
Blanco Lechoso	BL	University of Seville	Spain	Castellana	Cas	University of Seville	Spain
Pedrosillano	Ped	University of Seville	Spain	Pardina	Par	University of Seville	Spain
Sultano	S	University of Palermo	Italy	Laird	Lad	University of Palermo	Italy
Vulcano	V	University of Palermo	Italy				

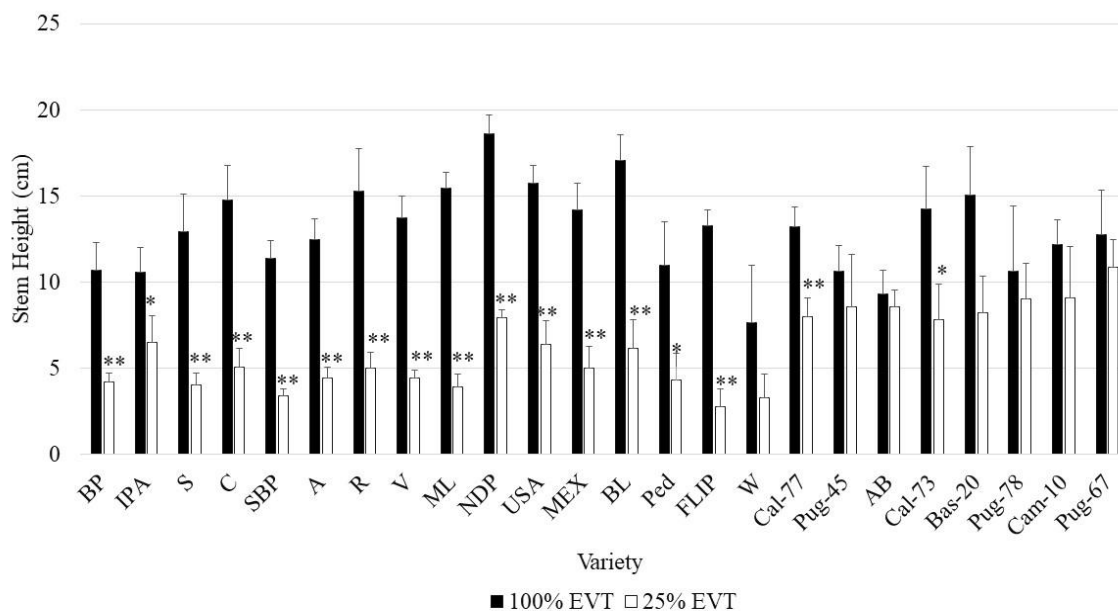


Figure 1. Chickpea cultivars and accessions used in the study.

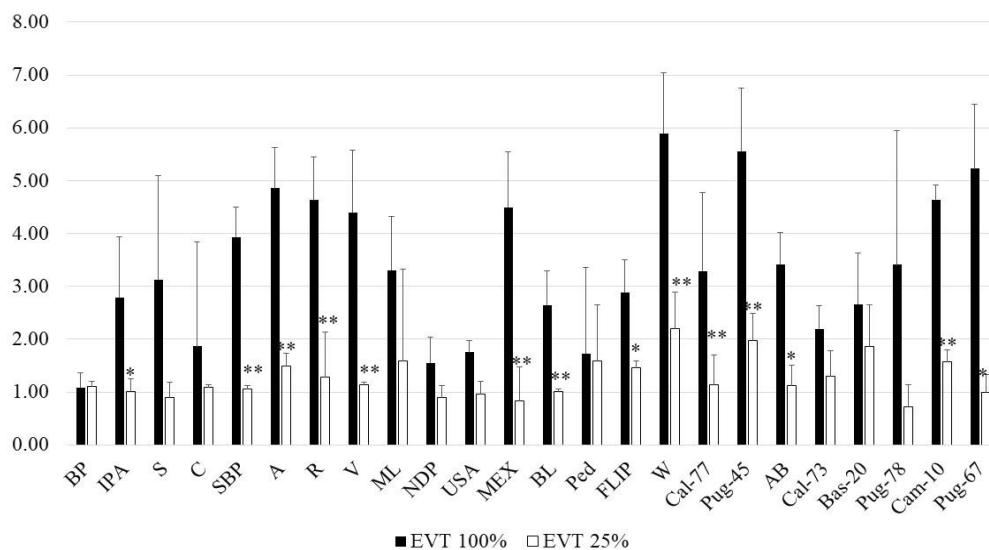


Figure 2. Lentils cultivars and accessions used in the study.

Results and discussion

Response of chickpea plants to water deficit under heat stress

In this study chickpea plants were exposed to different water availability (100% and 25% of EVT) under heat stress during the early vegetative stages to identify differential phenotypic response of the tested accessions. Figure 3 shows the average stem growth rate after 90 days post sowing based on normalized values against stem length at the beginning of the experiment (day 60) for each accession.

Under conditions of heat stress and limited water availability, chickpea cultivars exhibited a notable reduction in growth. This was also observed in accessions from the USA and Mexico, as well as in some of the Italian genotypes that were tested. The reduction of growth in some cases was severe, over 50% of reduced growth was recorded for all four cultivars as well as for accessions such as

NDP, ML, R, SBP6, BP and FLIP. The screening showed that Bas-20; Pug-57; Pug-28; Pug-45; Cam-10 have a good stem elongation even under reduced water availability, with no statistical differences to the growth of 100% EVT watered plants (Figure 3) demonstrating a good tolerance of water deficit under heat stress.

Chickpeas response to drought stress is influenced by the length and intensity of the stress, regardless of whether the stress is isolated or combined with other stressors. Drought in vegetative development stage of chickpea imposes restrictions on plant size and leaf area photosynthesis rate thus reducing growth and yield in maturation phase (Navkiran et al., 2014), especially when this stress is combined with high temperatures as demonstrated in this study. A significant decrease of stem height has been correlated with enhanced drought stress in canola (Khodabin et al., 2020).

In this plant species, a severe decrease of plant height in response to reduced water supply was correlated to lower photosynthesis rate as consequence of reduced photo-assimilates

production resulting in decreased plant organ in development. This may be due to abscisic acid change that affects shoot growth due to the reduction of protons secretion in the crosstalk with auxins (Rao & Mendham, 1991). The effect of drought on stem height growth rate is particularly evident at the end of vegetative growth, and close to flowering stage (Khalid & Ahmed, 2017) due to cumulative negative effects of drought on photosynthesis.

Heat stress combined with low water availability induced a reduction of biomass for most of the tested chickpea samples (Figure 4). Decrease of biomass production was not only result of

decreased stem elongation but also changes in water content influencing fresh/dry mass ration. Similarly to what observed for the stem height trait, biomass parameter showed high differences between accessions as well as cultivars under both 100% EVT and 25% EVT condition. Plant biomass is a parameter largely influenced by water availability and either water excess or deficiency can negatively affect it (Levitt, 1980). Biomass reduction was not always correlated to height reduction, in some cases even though height is reduced, plants retain the biomass production due to formation of more branches without affecting the height.

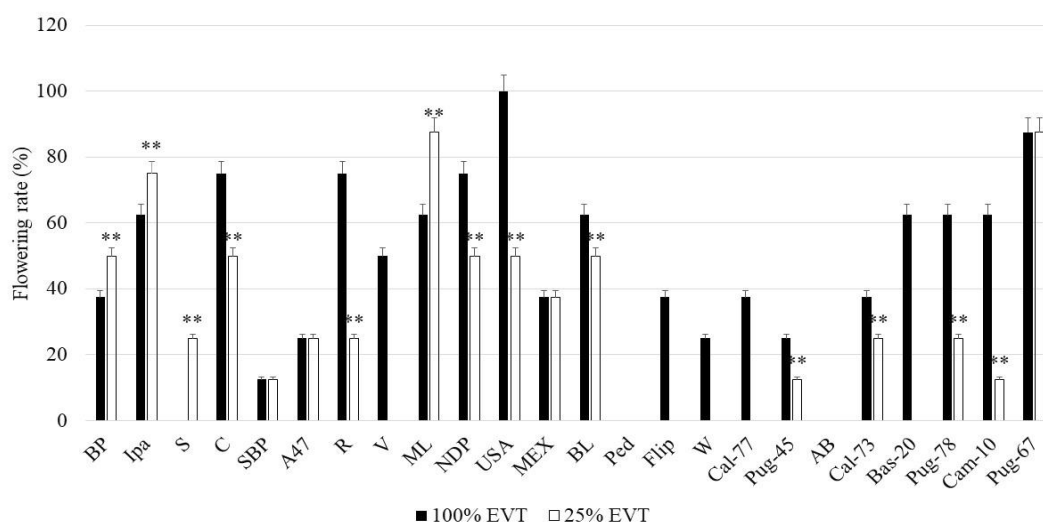


Figure 3. Stem elongation rate in chickpea under 100% and 25% EVT conditions. Values in the graph represent the values at day 90 after 4-week exposure to different irrigation. Values are normalized according to the start length at the beginning of the experiments. *,** Significant at $p < 0.01$ and 0.001 respectively. BP: Bussetto Palazzuolo; IPA: Ipa I2 Casteltermini; S: Sultano; C: Caltavuturo; SBP: San Biagio Platani Nero; A: Alcamo 47; R: Rosso; V: Vulcano; ML: Monteleppe; NDP: Nero di Puglia; USA: PI518248; MEX: PI572491; FLIP: Flip 08160C; W: W 69484; Cal-77: 111977 Ita/Calabria; Pug-45: 103245 Ita/Puglia; Cal-73: 111973 Ita/Calabria; Bas-20: 190222 Ita/Basilicata; Pug-78: 103278 Ita/Puglia; Cam-10: 110410 Ita/Campania; Pug-67: 103267 Ita/Puglia; BL: Blanco Lechoso; Ped: Pedrosillano.

Previous studies investigating the impacts of climate change on ecosystems, used a meta-analysis to determine the effects of drought on plant biomass production. The approach considered any potential constraining factors and demonstrated that aboveground biomass was partially affected to imposed drought events as well as to natural droughts. These results showed an underestimation of the effects of climate change impacts and highlight the requirement of integration of different approaches (Kröel-Dulay et al., 2022). In our study we observed that some accessions (such as ML and Bas-20) showed more consistent biomass production, even under drought

indicating that cultivation of different varieties can counteract drought effects by exploiting different tolerance levels in chickpea (Singh et al., 2022b). Combining heat stress with water deficit affected in different manner different genotypes, demonstrating that some genotypes are more sensitive to biomass reduction due to decreased water, while others are more affected by heat stress and have a reduced biomass production regardless of water availability. The negative effect of the drought was also reflected on flowering rate resulting with a significant reduction or complete absence of flower formation in most accessions of chickpea (Figure 5). Moreover, the transition from

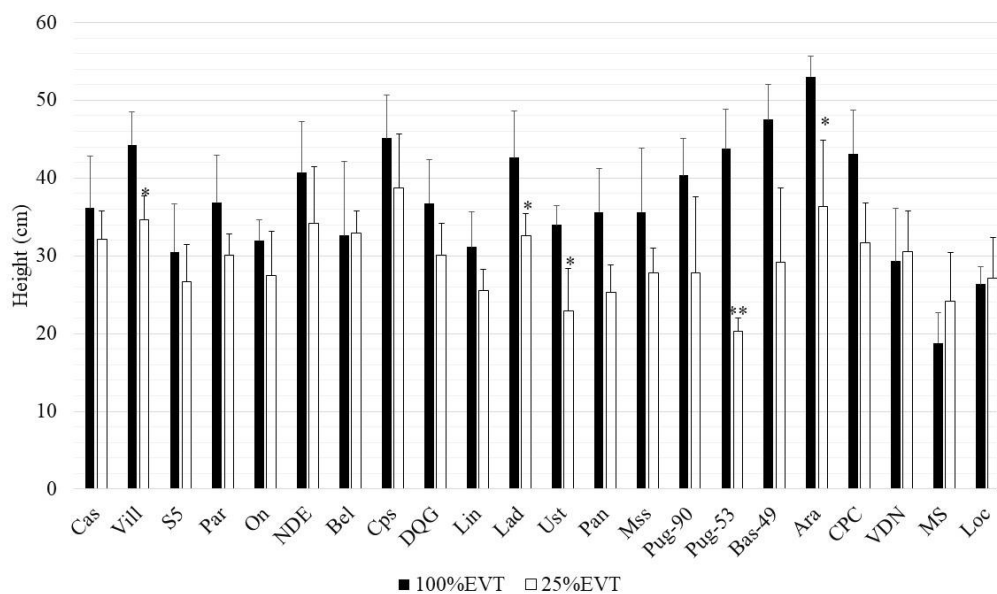


Figure 4. Dry mass of chickpea plants under 100% and 25% EVT conditions. Values in the graph represent the values at day 90 after 4-week exposure to different irrigation. *,** Significant at $p < 0.01$ and 0.001 respectively. BP: Bussetto Palazuolo; IPA: Ipa I2 Casteltermini; S: Sultano; C: Caltavulturno; SBP: San Biagio Platani Nero; A: Alcamo 47; R: Rosso; V: Vulcano; ML: Montelepre; NDP: Nero di Puglia; USA: PI518248; MEX: PI572491; FLIP: Flip 08160C; W: W 69484; Cal-77: 111977 Ita/Calabria; Pug-45: 103245 Ita/Puglia; Cal-73: 111973 Ita/Calabria; Bas-20: 190222 Ita/Basilicata; Pug-78: 103278 Ita/Puglia; Cam-10: 110410 Ita/Campania; Pug-67: 103267 Ita/Puglia; BL: Blanco Lechoso; Ped: Pedrosillano.

vegetative to reproductive stages is highly influenced by heat, which contributes to a reduction in the apportionment of substances from the apical organs to the pods.

Some genotypes did demonstrate higher tolerance toward the drought in respect to flowering rate, such as Pug-67, where percentage of flower formation matched the flowering percentage of 100% EVT plants. For some accessions an early onset of flowering was noticed, which was very visible for genotype S producing flowers before

flower formation in 100% EVT plants as result of heat stress, but in the later stages no podding was noticed. High temperatures (above 30 °C) during flowering can reduce pollen formation subsequently reducing pollination, fertilisation, and pod formation (Devasirvatham et al., 2012). Heat stress can also lead to loss of flower buds resulting in complete absence of flowering in chickpea varieties sensitive to drought and high temperatures (Devasirvatham and Tan, 2018).

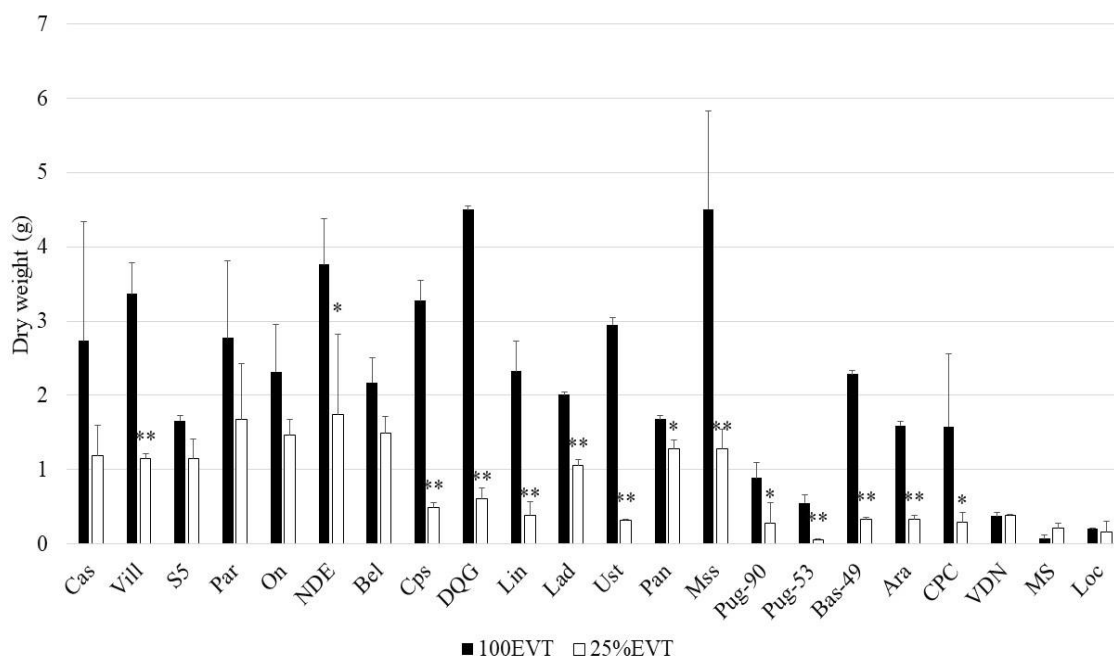


Figure 5. Flowering rate of chickpea genotypes under different water availability and heat stress. Values in the graph represent the values at day 90 after 4-week exposure to different irrigation. BP: Bussetto Palazzuolo; IPA: Ipa I2 Casteltermini; S: Sultano; C: Caltavuturo; SBP: San Biagio Platani Nero; A: Alcamo 47; R: Rosso; V: Vulcano; ML: Montelepre; NDP: Nero di Puglia; USA: PI518248; MEX: PI572491; FLIP: Flip 08160C; W: W 69484; Cal-77: 111977 Ita/Calabria; Pug-45: 103245 Ita/Puglia; Cal-73: 111973 Ita/Calabria; Bas-20: 190222 Ita/Basilicata; Pug-78: 103278 Ita/Puglia; Cam-10: 110410 Ita/Campania; Pug-67: 103267 Ita/Puglia; BL: Blanco Lechoso; Ped: Pedrosillano.

Early onset of temperature and drought stress can completely diminish yield in varieties with early flowering as recorded for some autochthonous accessions in this study. Early flowering is normally favoured in cultivation systems due to their extended reproductive period resulting in high yield (Kushwah et al., 2021).

The high temperatures also induced an early senescence leading to yellowing of the plant and slowing down the growth rate under 100% EVT as well as 25% EVT. Plant senescence, a genetically driven process, is also under environmental influence which often leads to premature senescence in plants under environmental stress such as drought (Gurumurthy et al., 2022). Early or speeded up senescence can influence yield in chickpea and can be correlated to high temperatures which is further emphasized by limiting water availability. Dropping the level of soil moisture below 50% of field capacity excretes drought stress to chickpea (Saeed et al., 2016), which in our study demonstrated with high impact on plant senescence, early onset of flowering in some accessions and very small rate of pod filling. Reduction of yield was also noticed for 100% EVT plants, exposed only to high temperatures; indeed, previous authors demonstrated that climate condition above 30°C affected the flowers survival by around the half (Kalve and Tadege, 2017), but combining heat stress with low moisture additionally reduced plant's ability for survival for most genotypes. Senescence dynamics in chickpea can be correlated to pod filling and grain yield and timing of the senescence onset is of crucial importance, whether the senescence is induced during stem elongation or flowering (Xie et al., 2016).

Response of lentil plants to water deficit under heat stress

Lentils showed higher tolerance to drought compared to chickpea, but tested accessions were susceptible to high temperature and overall stem elongation was reduced. Figure 6 shows the average stem growth rate 90 days after sowing based on normalized values against stem length at the beginning of the experiment for each accession.

The average stem elongation rate of the 22 tested accessions under the 100% of EVT irrigation ranged from 53.00 cm (Ara accession) to 18.80 cm (MS accession). No significant reduction of stem height was recorded for most accessions with only 9 out of 22 classified as sensitive to drought. Under water deficit conditions, the average elongation spanned from 38.75 cm (Cps) to 20.29 cm (Pug-53). Accessions VDN and Loc demonstrated similar stem elongation under both conditions (100 and 25% EVT) suggesting that these two could be classified as drought resistant.

Dry mass production was much more affected by the water availability demonstrating decrease of dry mass production at the end of the trails (Figure 7). Most of the accessions that showed differences in stem length under the differential EVT condition tested, also demonstrated a significant decrease of biomass under 25% EVT compared to 100% EVT.

High temperature induced significant differences between accessions in dry mass production with some accessions remaining high dry mass production under heat stress.

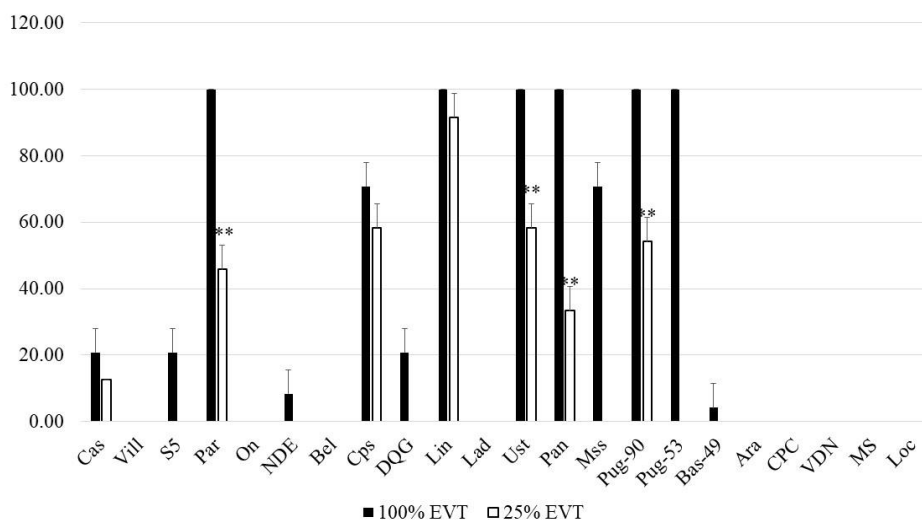


Figure 6. Stem elongation rate in lentils under 100% and 25% EVT conditions. Values are normalized according to the start length at the beginning of the experiments. Values in the graph represent the growth rate from day 60 to day 90 during exposure to different irrigation. *,** Significant at $p < 0.01$ and 0.001 respectively. Cas: Castellana; Vil: Villalba; S5: SFAX 5; Par: Pardina; On: Onano; NDE: Nera di Enna; Bel: Belliga; Cps: Campisi; DQG: Di quattro grande; Lin: Linosia; Lad: Laird; Ust: Ustica; Pan: Pantelleria; Mss: Mussomeli; Pug-90: 110290 Ita/Puglia; Pug-53: 103653 Ita/Puglia; Bas-49: 111849 Ita/Basilicata; Ara: Aragona Sicilia; CPC: Capracotta; VDN: Val di Nevola; MS: Monti Sibillini; Loc: Locale Puglia

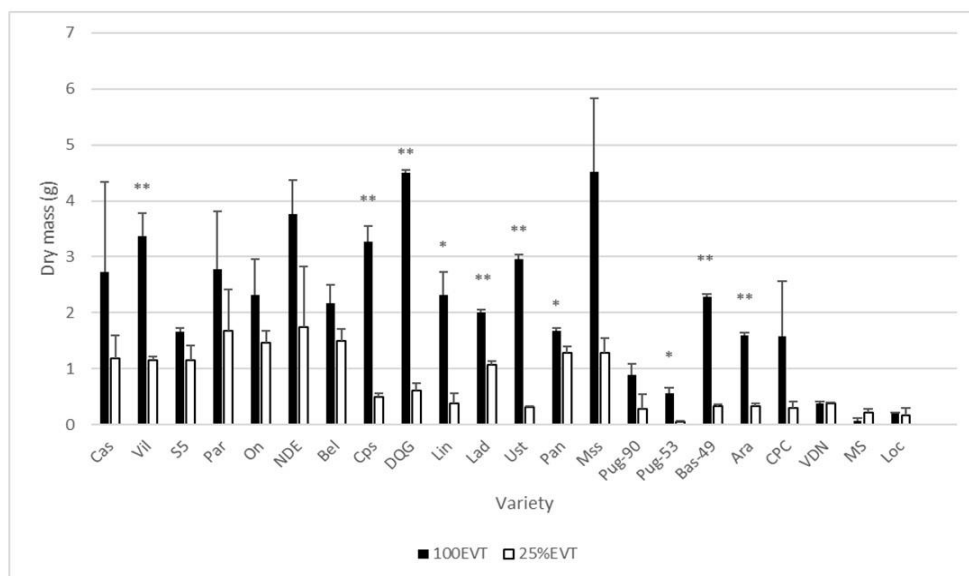


Figure 7. Biomass production in lentils under 100% and 25% EVT conditions. Values in the graph represent the growth rate from day 60 to day 90 during exposure to different irrigation. *,** Significant at $p < 0.01$ and 0.001 respectively. Cas: Castellana; Vil: Villalba; S5: SFAX 5; Par: Pardina; On: Onano; NDE: Nera di Enna; Bel: Belliga; Cps: Campisi; DQG: Di quattro grande; Lin: Linosia; Lad: Laird; Ust: Ustica; Pan: Pantelleria; Mss: Mussomeli; Pug-90: 110290 Ita/Puglia; Pug-53: 103653 Ita/Puglia; Bas-49: 111849 Ita/Basilicata; Ara: Aragona Sicilia; CPC: Capracotta; VDN: Val di Nevola; MS: Monti Sibillini; Loc: Locale Puglia

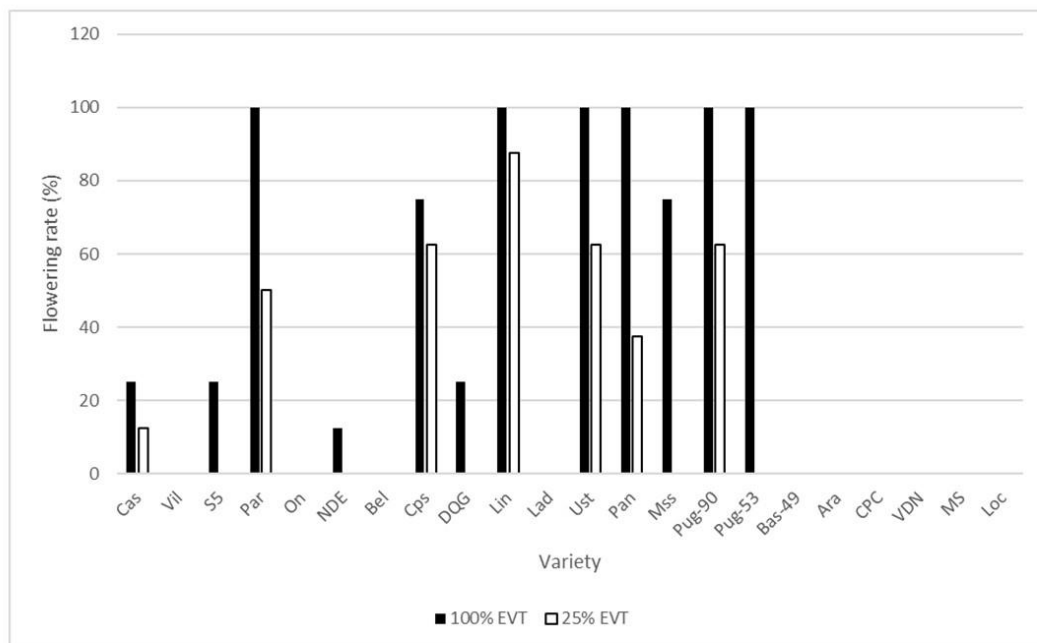


Figure 8. Flowering rate of lentils genotypes under different water availability and heat stress. Values in the graph represent the values at day 90 after 4-week exposure to different irrigation. Cas: Castellana; Vil: Villalba; S5: SFAX 5; Par: Pardina; On: Onano; NDE: Nera di Enna; Bel: Belliga; Cps: Campisi; DQG: Di quattro grande; Lin: Linosia; Lad: Laird; Ust: Ustica; Pan: Pantelleria; Mss: Mussomeli; Pug-90: 110290 Ita/Puglia; Pug-53: 103653 Ita/Puglia; Bas-49: 111849 Ita/Basilicata; Ara: Aragona Sicilia; CPC: Capracotta; VDN: Val di Nevola; MS: Monti Sibillini; Loc: Locale Puglia.

Imposing additional stress of drought affected also heat tolerant varieties demonstrating significant reduction of dry mass under 25% EVT conditions. Most of the tested accessions were sensitive to heat as well as drought with reduced or absent flowering followed with no podding (Figure 8). Exposing lentil plants to high heat during reproductive stage even few days limits photosynthesis resulting in low dry mass and causes flower abortion (Delahunty et al., 2015). High heat can also accelerate leaf senescence influencing seed weight and reducing the plants yield (Wahid et al., 2007). An example of how a high temperature can drastically effect lentils yield has been seen in 2009 when a heat wave in

Australia caused a 70% reduction of lent yield (Delahunty et al., 2015).

Conclusion

Exposure to drought during stem elongation and flowering can have a deuterating effects on yield of autochthonous and cultivated varieties of chickpea and lentil as demonstrated in this study. However, large scale screening of legume germplasm could be a valuable tool to identify new varieties that could show phenotypical traits more adaptable to climate related environmental stresses. To improve the reproductive efficiency and better success in chickpeas and lentils cultivation under adverse onditions associated to

climate change an extensive breeding effort should be focused on investigation of more tolerant genotypes and cultivation of more than one genotype in different crop systems. The genetic asset of the varieties most tolerant to heat and drought, and the microorganism's relationship under stressful environmental conditions should be investigated to better understand the most suitable genotypes for breeding purposes of these important legumes.

Germplasm variability is limited due to unwillingness of farmers to cultivate exotic genotypes giving a value to presented research. Sources of germplasm can be a good source for obtaining a drought tolerant genotype as demonstrated in the presented study.

Authors' contributions

Federico Martinelli carried out the study conception and design. **Angela Rosa Piergiovanni** provided genotypes and reviewed the first draft of the manuscript. Material preparation and data collection were performed by **Chiara Vergata**, **Francesco Caleri**, **Mattia Calvani** and **Federico Martinelli**. Analysis was performed by **Chiara Vergata** and **Erna Karalija**. The first draft of the manuscript was written by **Chiara Vergata** and **Erna Karalija** and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

Authors declare no conflict of interest.

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