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Comparative study of seed germination, phenology, and reproductive fitness in *Melilotus sulcatus* populations from Israel

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Abstract

Background *Melilotus sulcatus* (Fabaceae) is an annual weed species prevalent in the Mediterranean Basin and Asia, characterized by a hard seed coat, indicating physical seed dormancy and long seed-bank longevity. This weed exhibits high phenotypic plasticity, thriving across various climatic regions in Israel. This study investigates seed germination of *M. sulcatus* populations under different temperatures. Moreover, we have studied the phenology, and reproductive biology of *M. sulcatus* populations in a common garden experiment. Seeds of *M. sulcatus* were collected from onion fields at Kibbutz Grofit located in the hyper-arid Southern Arava region, with ~ 30 mm average annual rainfall, and from Kibbutz Yifat, in the Jezreel Valley, where annual rainfall is 450–650 mm. Progeny generations were grown in pollen-proof cages at Newe Ya'ar Research Center also located at the Jezreel Valley.

Results Differences in seed area and average weight were observed among populations and generations, with field populations producing heavier seeds compared to progeny populations. The optimal temperature (T_0) for seed germination varied among populations, with the highest T_0 recorded for the Grofit field population (23.40℃) collected at the warm site and the lowest for the Yifat field population (17.67℃) collected at the cold site, while the two progeny populations showed similar T_0 values. Phenology and reproductive biology were tested using seeds of the two field populations. Despite the vigorous growth of the Grofit population, there were no significant differences in average final weight (4.59 g for Grofit and 4.23 g for Yifat) and height (51.8 cm for Grofit and 50.3 cm for Yifat) between populations. Grofit progeny plants exhibited a statistically significantly higher number of seeds per pod, combined with larger seed area and average weight.

Conclusions Overall, our findings underscore the adaptive strategies employed by *M. sulcatus* populations in response to their environment. Vigorous plant growth, a higher number of seeds per pod, and larger seed area and average weight were observed in individuals from the Grofit population compared to the Yifat population. The greater fitness of Grofit population, originated from hyper-arid conditions, highlights the significant impact of environmental factors on species adaptation.

Keywords Reproductive biology, Weed infestation, Plant development

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Introduction

Melilotus sulcatus is a winter annual weed from the Fabaceae family, prevalent in the Mediterranean basin and Asia [1]. In Israel, *M. sulcatus* grows during the winter and blooms from March to early June. Recently, this weed has become increasingly problematic in crop and vegetable fields, increasing crop lost and thus gaining significant importance for farmers nationwide. *M. sulcatus* is primarily found in Amaryllidaceae crops, such as onions and garlic, but has also been reported in carrots and other vegetables. Soil solarization is a common preplant practice in Amaryllidaceae crops, effectively controlling a variety of soil-borne pathogens and reducing the seed viability of different weed species [2]. Like many other Fabaceae species, *M. sulcatus* has an impermeable seed coat that contributes to its longevity in the soil seed bank [3]. Wild pea seeds are characterized by low germination rates, however, Abbo et al. [4] showed that scarification have increased total emergence. Rubin and Benjamin [5] found that *M. sulcatus* seeds remain viable after soil solarization, likely due to their hard seed coat.

Examining the germination and emergence capabilities of diverse weed populations of the same species is pivotal for elucidating their interference potential across various cropping systems and habitats. Seed dormancy can affect germination time and the ability to remain viable in the soil for extended periods. Weed seeds may exhibit different dormancy and vitality characteristics, influencing their persistence and competitiveness in various agricultural settings [6, 7]. Both environmental factors and genetic traits can influence variations in seed size and weight, resulting in different emergence capabilities under various environmental conditions. For instance, *Parthenium hysterophorus* plants germinated from seeds collected in multiple locations and grown under unlimited resources (greenhouse conditions) produced seeds with higher seed weight compared to seeds collected from the same population in the field. This suggests that favorable growing conditions may lead to increased reproductive investment and influence seed traits [8]. In *Solanum elaeagnifolium*, a temperature-based model presented similar germination patterns across populations that were collected in different locations [9]. Conversely, *Amaranthus palmeri* demonstrated different germination capabilities between populations when assessed using a hydrothermal model [10]. For instance, seeds produced under water stress demonstrated a higher germination rate under low water potentials compared to seeds from beneficial irrigation conditions. This suggests that individuals grown in harsher climates may exhibit resilience to similar environmental conditions in future generations. Modeling germination under various environmental conditions may assist in understanding the significant impact of environmental factors on population adaptation.

Ecological control tools rely on data regarding key phases in a weed's life cycle and phenology [12]. A previous study has shown that some *Melilotus* spp., including *M. sulcatus*, can grow under salinity and waterlogging regimes, indicating tolerance to saline soils [13]. However, to the best of our knowledge, temperature-based conditions for germination have not been examined for this species. For its close relative, *M. officinalis*, maximum germination was obtained between 15 and 30 °C [14]. *Melilotus* species possess seed physical dormancy, primarily caused by an impermeable seed coat that prevents water uptake. Seasonal temperature fluctuations may help regulate dormancy release, with warm or fluctuating autumn temperatures breaking dormancy and preparing seeds to germinate during winter season [3]. Additionally, the synergistic effects of desiccation and temperature fluctuations can contribute to the breakdown of physical dormancy, ensuring that germination occurs under optimal environmental conditions [15]. Key information describing the phenology and reproductive capabilities of *M. sulcatus* is scarce, hindering a comprehensive understanding of its fitness under different environmental conditions.

Melilotus sulcatus is considered as a major weed in Israel especially in two climatic areas, in the hyper-arid Southern Arava region, with ~ 30 mm average annual rainfall, and in the Jezreel Valley where annual rainfall is 450–650 mm. Thus, seed populations used in this study were collected in Kibbutz Grofit (Southern Arava), and Kibbutz Yifat (Jezreel Valley). The focus of our study is on *M. sulcatus* as a weed species infesting agricultural fields across diverse climatic regions. Therefore, water is unlikely to be the primary limiting factor for germination for the collected seed populations; instead, temperature plays a more critical role. The objectives of this study were to (i) examine the germination of *M. sulcatus* populations under different temperatures. In addition, we (ii) examine plant development, phenology, and reproductive capabilities through a common garden experiment. For this purpose, we recorded seed shape and average weight parameters and investigated germination under different temperatures. Subsequently, a net house experiment was conducted to examine phenological differences and reproductive output among seed populations.

Results

Seed size and average weight

The examination of seed area across the two parental and two progeny populations revealed significant differences between parental and progeny populations. Specifically, seed area was significantly higher in Grofit compared to Yifat for both the field (3.47 ± 0.62 mm^2

vs. 2.39±0.32 mm²) and progeny populations (3.36±0.59 mm² vs. 2.47±0.26 mm²), with $p<0.001$ for both (Fig. 1A). However, no significant differences in seed area were observed between the field and progeny generations within each population.

Significant differences in seed average weight were also observed across all comparisons. Grofit exhibited heavier seed weight than Yifat in both the field (0.21±0.02 g vs. 0.18±0.005 g, $p<0.001$) and progeny populations (0.20±0.01 g vs. 0.14±0.002 g, $p<0.01$) (Fig. 1B). Additionally, seeds from the parental populations were consistently heavier than those from their progenies, with statistical significance observed for Grofit ($p<0.05$) and Yifat ($p<0.001$).

Germination under different temperatures

Seeds of *M. sulcatus* germinated at all tested temperatures (5–30 °C) for both field-collected seeds and their progenies (Fig. 2). Germination occurred as early as 12 h after sowing, with faster rates observed under 15 °C and 20 °C.

The maximum temperature for germination (T_c) was higher for field populations of both Grofit (42.9 °C vs. 29.71 °C) and Yifat (37.14 °C vs. 29.70 °C) compared to the progeny populations (Table 1). The base temperature for seed germination (T_b) was lower for the field populations compared to the progenies, with the Yifat field population showing the lowest T_b of -1.8 °C. Both progeny populations had similar T_b values: 7.12 °C for Grofit and 7.37 °C for Yifat. Comparing the optimal temperature (T_0) for seed germination, the parental populations had higher T_0 for Grofit (23.40 °C vs. 18.41035 °C) but not for Yifat (17.67 °C vs. 18.54 °C) population compared to their progeny.

Development and phenology

Plant development for both field populations was evaluated under net house conditions by measuring weekly height increments. The Grofit population exhibited a significantly greater growth rate in absolute height per week compared to the Yifat population throughout the experiment (Fig. 3A). For example, at 14 days after transplanting (DAT), Grofit plants reached an average height of 3.64±0.45 cm, while Yifat plants averaged 2.48±0.64 cm. The largest disparity in plant height was observed at 49 and 56 DAT, where Grofit plants attained an average height of 18.37±3.13 cm and 26.06±3.65 cm, respectively, compared to 12.79±2.51 cm and 19.47±2.90 cm for Yifat plants (Fig. 3A). Despite differences in growth rates observed throughout the experiment, the final average height measured after 70 days did not show significant differences between the two populations (Fig. 3B).

Reproduction biology

In *M. sulcatus*, flowering initiates with the appearance of the flowering bud and progresses to anthesis, the stage when the flower is fully open and functional. We documented the timing of these reproductive stages. In

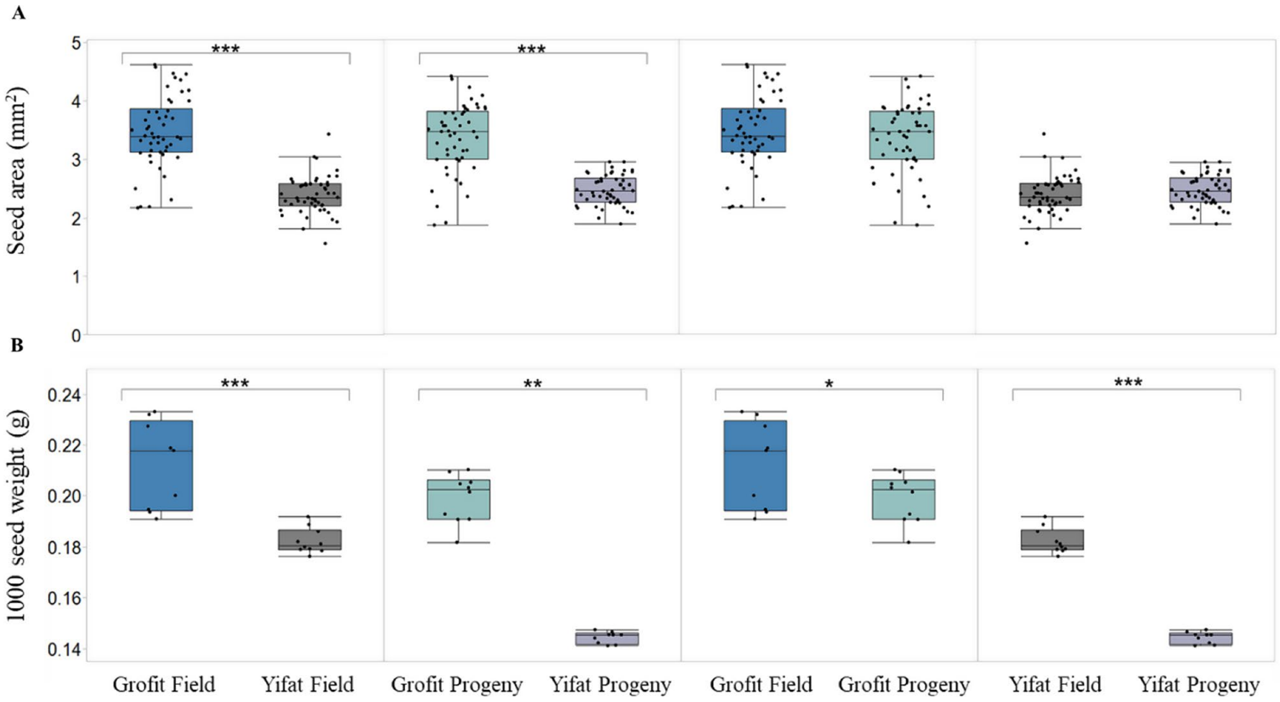

Fig. 1 Seed spatial parameters and average weight of field and progeny populations of *M. sulcatus* (Grofit and Yifat). Seed area (mm²), $n=50$ (**A**). Seed average weight (g), $n=10$ (**B**). Differences among populations were tested using *t*-test (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

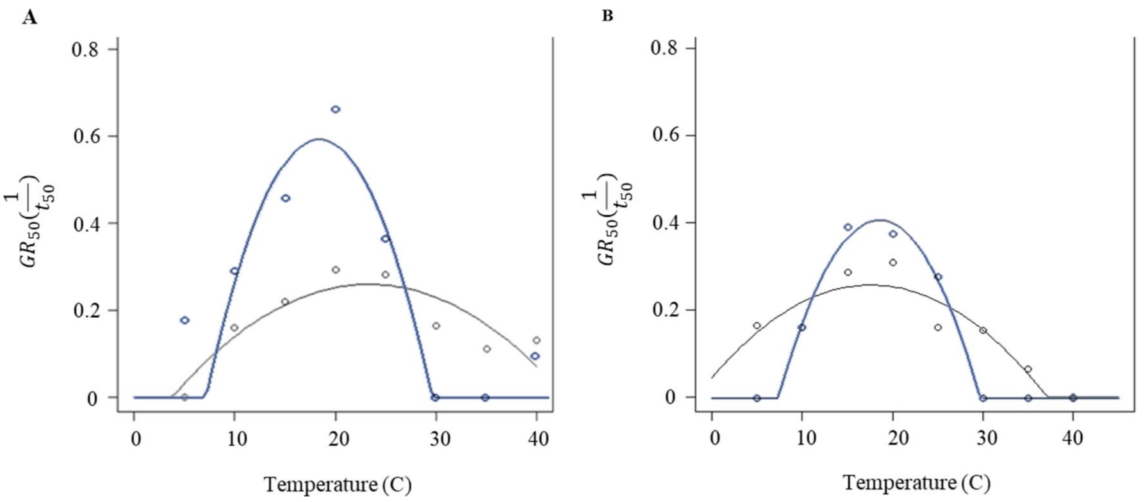

Fig. 2 A polynomial temperature effect model estimating 50% germination rate (GR_{50}) at different temperatures for field (grey line) and progeny (blue line) populations of *M. sulcatus* (**A** - Grofit, **B** - Yifat). Model fit was attained using a polynomial temperature effect equation (equation B, materials, and methods), $n = 25$. RMSE; Grofit field (0.004) and progeny (0.0001), Yifat field (0.003) and progeny (0.001)

Table 1 Parameters of the polynomial temperature effect for germination of *M. Sulcatus* field and progeny populations under different temperatures. θ_T- thermal time for seed germination, T_c- maximum temperature for seed germination, T_b- base temperature for seed germination, T_o- optimal temperature for germination

	Parameter	**Θ_T**		**T_c**		**T_b**		**T_o**	
Population/Generation		**Field**	**Progeny**	**Field**	**Progeny**	**Field**	**Progeny**	**Field**	**Progeny**
Grofit	Estimate	37.65	9.54	42.92	29.71	3.80	7.12	23.40	18.41
	Std. Error	6.38	2.74	2.33	2.65	1.95	1.86		
	p-value	0.001	0.017	8.66e-06	9.80e-05	0.11	0.012		
Yifat	Estimate	37.84	13.71	37.14	29.70	-1.80	7.37	17.67	18.54
	Std. Error	7.41	0.85	2.10	0.60	3.40	0.39		
	p-value	0.004	1.64e-05	9.35e-06	5.29e-08	0.62	7.47e-06		

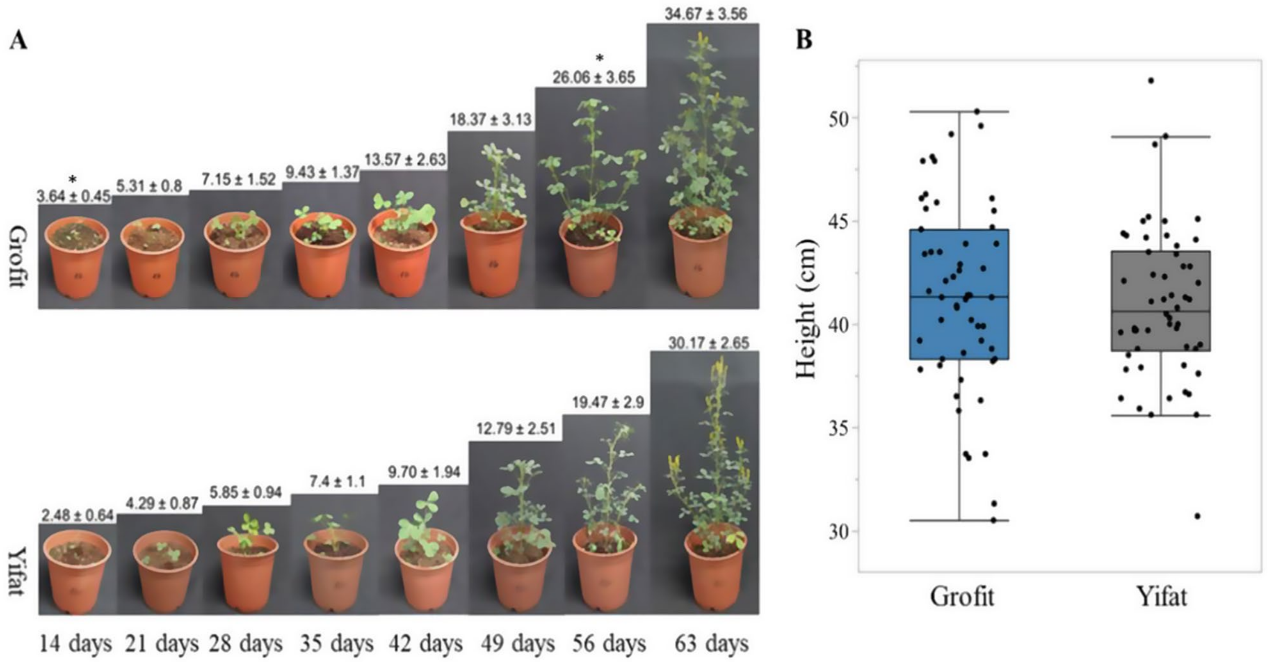

Fig. 3 Differences in growth rate among the two field populations of *M. sulcatus*. Weekly height comparison of the two populations of *M. sulcatus* plants (cm ± SE) (**A**). Final height of *M. sulcatus* plants 70 days after transplanting ($n = 54$) (**B**). Statistical differences were tested using *t*-test ($^*p < 0.05$)

the Grofit population, two distinct phenotypic groups were observed for the onset of flowering bud appearance: one subset began flowering as early as 34 days after transplanting (DAT), while most plants initiated flowering around 50 DAT (Fig. 4A). Most flowering buds in the Grofit population emerged between 50 and 63 DAT. Similarly, variable phenotypic responses were noted for anthesis in the Grofit population compared to a more uniform response observed in the Yifat population (Fig. 4B).

To explore the variation in timing between first bud appearance and anthesis in both populations, we generated a density plot (Fig. 4C). The median time from flowering (inflorescence visible) to anthesis was longer in the Yifat population (7.53 days) compared to the Grofit population (5.98 days). However, the overall trend indicates greater uniformity among Yifat plants compared to Grofit plants, as evidenced by the similar shape of the curve between the two events (flowering and anthesis). Notably, both the appearance of the first flowering bud and the onset of anthesis occurred earlier in the Grofit population.

Plant productivity

The assessment of plant productivity for both populations involved measuring final plant dry weight, pod number per plant, and seed production per pod (50 pods per plant). Despite the differences in growth rates observed throughout the experiment, the average dry weight measured at the end of the experiment was similar for both populations (Grofit: 4.23±0.91 g; Yifat: 4.59±0.95 g; Fig. 5A).

The number of pods per plant was recorded for 10 randomly selected plants from each population, providing insights into their reproductive capacity and potential weed infestation potential. While there was variation

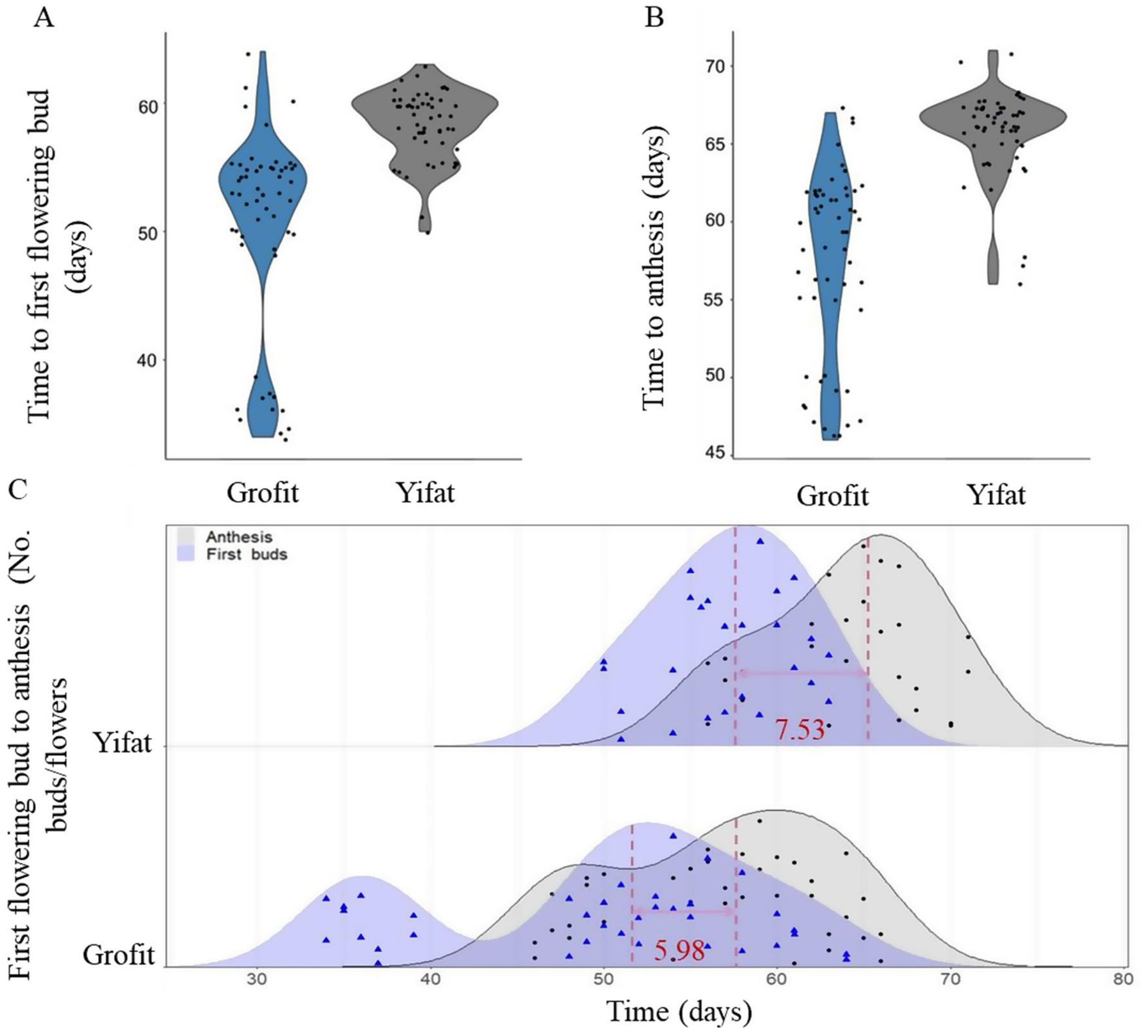

Fig. 4 Time to flowering in *M. sulcatus* field populations. Time for initiation of the first bud (**A**) and time to anthesis (**B**). Distribution plot showing time to first bud/anthesis of *M. sulcatus* plants for each population (**C**). The Median time (days) for each parameter is presented as a dashed line: Grofit 51.54 (first bud), 57.52 (anthesis); Yifat 57.5 (first bud), 65.03 (anthesis), *n* = 54

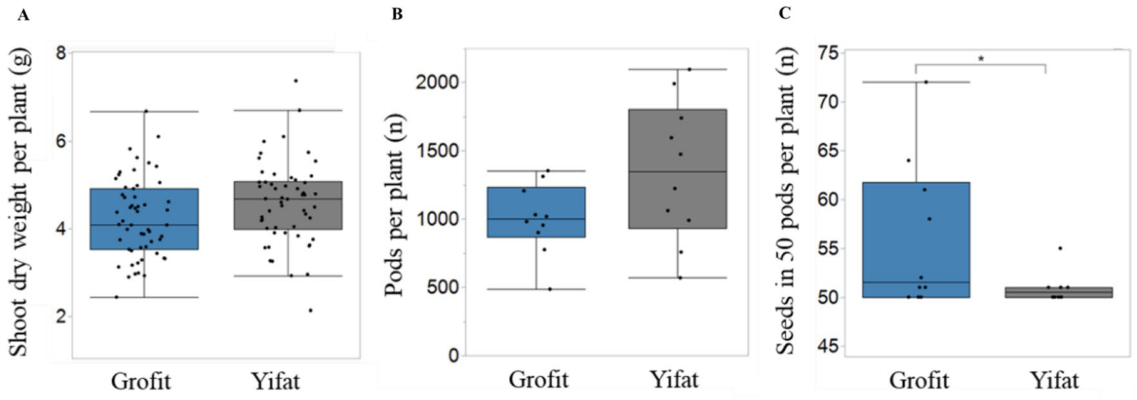

Fig. 5 Differences in Plant productivity among field populations. Shoot dry biomass ($n = 54$) was recorded for plants of Grofit and Yifat populations at the end of the experiment (**A**). The number of pods per plant (**B**) and seeds counted for 50 pods (**C**) were recorded for 10 plants from each population. Statistical differences were tested using *t*-test (*$p < 0.05$)

among the populations, the average number of pods per plant did not show significant differences between the Grofit population (1001.7±257.14) and the Yifat population (1415.56±499.44; Fig. 5B). However, it is noteworthy that plants from the Yifat population tended to produce a higher number of pods per plant compared to those from the Grofit population. Some individual plants produced up to 2000 pods, whereas pod production for Yifat plants did not exceed 1500 pods per plant (Fig. 5B).

Preliminary observations indicated variability in pod seed content, with some pods containing two seeds and others containing only one. Threshing 50 pods individually revealed that a larger proportion of pods from the Grofit population contained two seeds compared to the Yifat population (Fig. 5C; $p < 0.05$).

Discussion

In our study, we examined the germination dynamics, phenology, and reproductive biology of *M. sulcatus*. Seeds from different regions showed notable differences in size and average weight (Fig. 2). Specifically, seeds collected from the southern location (Grofit) were significantly larger and heavier compared to seeds from the northern location (Yifat), a trend that persisted in the progeny populations as well. Overall, the average seed weight for all populations ranged from 0.14±0.002 g to 0.21±0.02 g. These results align with a study by Zhang et al. [16], which reported similar trends in seed weight for *M. sulcatus* seeds from Tunisia and Morocco, averaging 0.16 g and 0.23 g, respectively. These findings closely resemble the range of seed weights observed in our investigation.

Our investigation further revealed that seeds from the field populations were generally heavier than those from the progeny generation (Fig. 1). Seed weight in population of *Desmodium paniculatum* was found to be influenced by environmental conditions, such as high temperature, leading to reduced nutrient availability and lower seed weight [17]. Variations in seed size among plants of the same species grown in different climatic zones may indicate adaptation to environmental conditions [18]. For example, in pine species (subgenera *Strobus* and *Pinus*), seeds from arid environments tend to be heavier than those from cold environments [19].

One possible explanation for differences in seed area and average weight among *M. sulcatus* field populations is that seeds in hyper-arid regions require more initial resources for emergence and development to survive harsh environmental conditions. Similarly, studies on other plant species, like *Amaranthus retroflexus*, have shown that seeds from drier environments tend to be larger compared to those from wetter environments [20]. Leishman and Westoby [21] showed in glasshouse experiments that survival from emergence of seedlings of different plant species originated from large seeds increased under increasingly dry soil conditions in comparison to seeds originated from small seeds, moreover, survival time of seedlings under drying soil conditions was positively associated with seed size. This data suggests that environmental factors significantly influence seed characteristics in *M. sulcatus* and other plant species. The observed differences are mitigated through maternal effects, as transgenerational effects suggest that environmental conditions during seed filling may induce genetic or epigenetic changes influencing seed area and weight, impacting seed development and potential fitness in subsequent generations.

The examination of *M. sulcatus* seed germination revealed a wide temperature tolerance, ranging from 5 to 40°C. Variations in germination rates among populations were particularly notable at extreme temperatures (5°C, 35°C, and 40°C). For the field populations, the optimal temperature for germination (T_0) was higher for Grofit compared to Yifat (23.40°C vs. 17.67°C, respectively;

Table 1), whereas for the progeny populations, T_0 values were similar between Grofit and Yifat (18.41°C vs. 18.54°C, respectively; Table 1). Additionally, the base temperature (T_b) for the Yifat field population was lower than that of the Grofit field population (-1.80°C vs. 3.80°C, respectively; Table 1). Moreover, Grofit progeny seeds, which were grown under the northern region conditions (Newe Yaar Research Center), showed similar T_0 values to the Yifat field and progeny populations (Table 1). The strong environmental effect is evident in the observation that each field population displays a different optimal temperature for germination. However, when seeds from both populations are grown under the same conditions, even for just one generation, their germination optimal temperature becomes similar. This highlights the substantial influence of environmental conditions on seed characteristics and adaptation. These findings also suggest that the environmental conditions experienced by the mother plants strongly influence the germination characteristics of the seeds. Similar studies in other plant species, such as *A. palmeri* and *P. hysterophorus*, have shown that seed germination traits can be influenced by parental environmental conditions and exhibit transgenerational plasticity [8, 10]. Our results indicate that seed parameters, average weight, and germination dynamics of *M. sulcatus* populations demonstrate high plasticity, with environmental conditions significantly affecting these traits across generations.

The genetic basis of flowering in response to environmental cues has been widely discussed [22]. Numerous publications have highlighted how environmental conditions can influence flowering time across different plant species. For example, Scandinavian populations of *Arabidopsis lyrata*, originating from distinct climatic environments, exhibited variations in flowering time correlated with their environmental conditions [23]. Similarly, Iannucci et al. [24] explored the effect of temperature on flowering time across multiple Fabaceae species, demonstrating that higher temperatures generally lead to earlier flowering. In *Chamaecrista fasciculate*, seasonal fluctuations in temperature was found to be correlated with floral longevity and flower deployment [25]. Research on flowering in legumes suggests that environmental stress can both delay and hasten flowering, depending on the severity and timing of the stressor, highlighting the complexity of adaptation mechanisms across species [26].

In our study, we observed distinct patterns in the flowering behavior of *M. sulcatus* populations. Plants from the Yifat population exhibited a normal distribution of flowering times, whereas plants from the Grofit population displayed two distinct cohorts of early and late flowering individuals. The majority of Grofit plants flowered earlier, with flowering extending up to 70 days, whereas Yifat plants had a broader range, with anthesis occurring up to 80 days. Notably, both populations were grown under identical environmental conditions, suggesting that genetic differences play a significant role in determining flowering time in *M. sulcatus*.

This variability in flowering time between populations likely reflects genetic differences that interact with environmental cues, known as genotype-by-environment (G×E) interactions. The presence of two cohorts of early and late flowering within the Grofit population further supports the hypothesis that genetic diversity influences flowering dynamics in *M. sulcatus*. Genetic variation and phenotypic plasticity are common in weed species, facilitating adaptation to diverse environments [27, 28]. Further investigation is warranted to elucidate the genetic mechanisms driving these differences in flowering time and how these mechanisms interact with environmental factors.

Seed production is a significant indicator of weed population fitness [29, 30]. Our study on the reproductive output of two *M. sulcatus* field populations revealed distinct patterns. Plants from the Yifat population produced a higher number of pods (Fig. 5B), while plants from the Grofit population yielded a greater number of seeds per pod when counted across 50 pods per plant (Fig. 5C). Additionally, seeds from both the field and progeny populations of the Grofit population were larger and heavier compared to those of the Yifat population (Fig. 1). This combination of larger seed size, higher seed average weight, and increased seed number per pod suggests greater fitness of the Grofit population.

Conclusions

Our study comprehensively characterized differences among *M. sulcatus* populations across various life stages, including seed germination, development rate, transition from vegetative to reproductive stages, flowering, and seed production. We observed transgenerational variation in seed germination responses to different temperatures, while seed area and average weight showed less variability. When examining plant development across these life stages within field populations, individuals from the Yifat population exhibited relative uniformity, whereas plants from the Grofit population displayed higher variation. This variability, particularly evident in flowering time across the Grofit population and the higher number of pods per plant and seeds per pod, suggests the potential operation of a bet-hedging strategy. The bet-hedging theory posits that plants from environments with fluctuating or extreme conditions intentionally produce offspring with diverse phenotypes, which may germinate at different times or exhibit varied traits. This strategy mitigates risks associated with unpredictable environmental fluctuations [31–33]. By germinating in groups over time, plants reduce competition between individuals and spread the risk over a longer period [34]. For *M. sulcatus*, variation in flowering time across the Grofit population, coupled with increased reproductive output, likely enhances the species' ability to survive and reproduce in the harsh hyper-arid conditions of the Arava region. Understanding the temperature range for *M. sulcatus* germination at different climatic regions may have implications for practical agricultural issues, increasing the efficacy of weed control measures. For instance, improving herbicide application timing or other control measures such as cultivation and harrowing, may reduce weed competition with crop plants. Data on weed flowering and seed production timing are also crucial as it enables farmers to act before weeds set seeds, preventing large-scale seed dispersal. This helps to reduce future weed infestations, and may lower weed pressure for subsequent crops.

Overall, our findings underscore the adaptive strategies employed by *M. sulcatus* populations in response to their environment. The greater fitness of the population originated from hyper-arid conditions highlights the significant impact of environmental factors on species adaptation.

Materials and methods

Seed collection & plant material

Melilotus sulcatus seeds were collected from two sites where farmers reported severe infestation: Southern Arava, Kibbutz Grofit (29°56'50.6"N, 35°04'20.8"E) at 2020, and Jezreel Valley, Kibbutz Yifat (32°38'13.4"N, 35°14'24.5"E) at 2021. Plant material is publicly available and will be send upon reasonable request to the corresponding author. Seeds of both populations were deposited in the Israeli Plant Gene Bank; Grofit seeds voucher number 10,030,437, Yifat seeds voucher number 10,030,436. The Southern Arava region has a hyper-arid climate, with ~ 30 mm average annual rainfall. The average daily winter temperature in the Arava region is 21 °C, while maximum daily temperatures in the summer reach an average of 44 °C. For the Jezreel Valley, summers are warm, with an average maximum daily temperature of 31 °C, and winters are characterized by average maximum temperatures of 17 °C, and annual rainfall is 450–650 mm (https://ims.gov.il/he). Seeds of the two *M. sulcatus* populations were collected from 30 to 40 mature plants haphazardly across the field. For each field population, progeny generation was reproduced after growing in pollen-proof cages placed in a net house under winter conditions (March-May 2022) at the Newe-Ya'ar Research Center (32°42'27.7"N, 35°10'48.3"E). Each cage accommodated 25–30 plants, watered daily and grown to maturity. Seeds were collected in bulk and designated as the progeny population.

Seed spatial parameters and weight

To evaluate the spatial parameters, 50 seeds from each field and progeny population were photographed using an electronic microscope (Hirox®, 3D digital microscope) equipped with a digital camera (CMOS high-definition sensor) and a zoom lens (MXB-050Z 50-400x {20- 800x}). A built-in image analysis software (RH 2000) was used to record the spatial parameters based on the surface area of each seed. For seed average weight, 1000 seeds were counted in 10 replicates of 100 seeds. Seeds were weighed on a micro-scale (Mettler-Toledo® GmbH, WXTE).

Seed germination

Seed germination dynamics were examined under constant temperatures in a growth chamber (Pol-Eko-Aparatura incubator ISO:9001, 2008). Seeds were collected from both fields (Yifat, Grofit), and their progeny seeds were grown at the Newe-Ya'ar Research Center. Seeds were first treated to break physical seed dormancy using the following protocol: seeds were rubbed with coarse sandpaper (AJR11, Karbosan©), immersed in 70% ethanol for one minute, and immersed in 2% sulfuric acid for 15 min. After this treatment, the seeds were thoroughly washed under running water.

For each temperature treatment, seeds were placed in 9-cm Petri dishes lined with filter paper (Whatman® Grade 2; Sigma-Aldrich) and sealed with Parafilm® to reduce water loss. We tested seed germination at eight constant temperatures: 5, 10, 15, 20, 25, 30, 35, and 40℃. Each combined treatment (population * temperature) was tested in four replicates with 25 seeds in each replicate. Germination was monitored in 12-hour intervals over 14 days.

To develop the temperature-based model, several equations were used:

The first was a log-logistic sigmoidal equation with five parameters.

$$f(x) = c + \frac{d - c}{(1 + \exp(b(\log(x) - \log(e))))^{f}} \tag{1}$$

Parameters shown in this equation are the slope in the inflection point (b), upper limit (d), lower limit (c), and infliction point (e). The infliction point describes where 50% of maximal germination occurred for each treatment. When the value of f equals 1 the equation is symmetrical; when different, it is non-symmetrical. Using this equation, the value of t_{50} was extracted, the time for 50% of maximal germination or the time for the e parameter value for each trial repetition. $t_{50}$was converted into germination rate (GR_{50}) for that percentile using the following equation .

$$GR_g = \frac{1}{t_g} \tag{2}$$

When g refers to the percentile used.

We also used the equation for the polynomial temperature effect model [8, 35] with three parameters.

$$GR = \frac{\max(T, T_b) - T_b}{\theta_T}\left[1 - \frac{\min(T, T_c) - T_b}{T_c - T_b}\right] \tag{3}$$

Parameters in this equation express the basic temperature (T_b), maximum temperature (T_c), and the thermal time for germination (θ_T). Using the values of T and GR_{50} of each repetition in equation C, all the above parameters are given.

Since this curve is symmetrical, the optimal temperature values was derived from the following equation.

$$T_o = \frac{T_c - T_b}{2} + T_b \tag{4}$$

Data analysis was performed using R (version 3.3.2, i386) in the RStudio (version 1.3.1056) integrated environment using the *drm*() function [36] and *GRT.M*() function for the polynomial temperature effect for a cardinal model [36].

Growth and development of *M. sulcatus*

The experiment was conducted at the Newe-Ya'ar Research Center in a net house under Mediterranean winter-spring conditions (February-May). Seeds from two field populations, Grofit and Yifat, were used. Seeds were germinated in the net-house and grown to the seedling stage. Then, seedlings were transplanted into 3 L pots filled with a commercial growth mixture (Tuf Marom-Golan®). For each population, 54 plants were used to describe phenological differences. Parameters recorded during the experiment included the day of flowering and anthesis. Plant height was measured weekly and upon study completion.

At the end of the experiment, the above-ground shoots were separated, cut, placed in paper bags, and dried in an oven for 48 h at 65℃. To assess differences in plant reproductive abilities among populations, pods from 10 plants from each population were harvested, and the number of pods per plant was counted. Seeds from each pod were then threshed and counted separately using a seed counter (DATA Counts S-25, DATA Detection Technologies Ltd.).

Data analysis

Statistical differences in seed morphological features, seed average weight, plant height, biomass, and reproductive output among populations were analyzed using One-way ANOVA Analysis with a *t*-test between every two populations, JMP® Pro 16 (SAS Institute Inc.). To evaluate flowering timing, flowering data were presented using R version 4.2.1 and RStudio; a density plot using the "ggridges" package and a violin plot using the "ggplot2" package [36].

Abbreviations

DAT	Days After Transplanting
GR_{50}	Germination Rate of 50%
T_b	Base Temperature for Seed Germination
T_0	Optimal Temperature for Seed Germination
T_c	Maximum Temperature for Seed Germination
θ_T	Thermal Time for Seed Germination

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Author contributions

M.M. and D.G. secured the funding for this project. M.M., A.G. and D.G. contributed to the study's conception and design. Data collection and analysis were performed by S.Z. and J.A-N. The first draft of the manuscript was written by S.Z. and M.M and was send to all authors. M.M., A.G. and D.G. supervised and administered the project. All authors read and approved the manuscript.

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Data availability

The datasets analyzed during the current study are available from thecorresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Seeds of *M. sulcatus* were sampled on private agricultural land with the explicit permission of the landowner.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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