



# Variability in germination fractions of *Amaranthus albus* in response to weed management and abiotic maternal conditions

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## ABSTRACT

Understanding the effects of abiotic, biotic, and management factors on weed germination is vital for optimizing weed control in agricultural fields. Nevertheless, the considerable variability among weed populations may undermine the reliability of these efforts. This study explores the intra-specific variability of *Amaranthus albus* populations obtained from agricultural fields across a climate gradient, ranging from semi-arid to Mediterranean. We explore the influence of temperature on germination, characterize *A. albus* seed morphology and assess seedling growth rate, while considering maternal effects. Nine populations were collected in northern Israel, and F<sub>2</sub> generation seeds were produced under uniform conditions. Seed traits, germination patterns and growth rate of both maternal and progeny populations were characterized and compared. The estimated parameters of time-to-event models of populations and generations were compared using a meta-analytic approach. There were significant seed weight differences among the F<sub>1</sub> populations, with eastern populations having the highest mean weights (28.6–38.5 mg per 50 seeds). Overall, F<sub>1</sub> populations had higher seed weight than F<sub>2</sub> populations (25.5 vs. 23.4 mg;  $p < 0.001$ ). Two distinct germination patterns were observed in F<sub>1</sub> populations: For the early-season planting areas, germination was rapid and uniform, with higher germination percentages at lower temperatures; in contrast, for the late-planting areas, germination was delayed and variable, with higher percentages under more favorable conditions (30 °C). F<sub>2</sub> populations exhibited nearly identical germination patterns in response to temperature. The initial growth rate over time was similar across populations and generations, indicating that maternal conditions had no effect beyond the germination phase. The plasticity in the germination response to temperature suggests that modeling *A. albus* behavior is possible but requires a thorough understanding of underlying system components. The study emphasizes the importance of incorporating location-specific factors into weed management strategies, considering both natural processes and anthropogenic influences shaping weed populations.

## 1. Introduction

Understanding the impact of environmental and management factors on the functional traits of weeds is crucial for effective and environmentally friendly weed management (Gaba et al., 2017; Holst et al., 2007). Substantial variability in functional traits has been recorded among populations of weed species, both on a large geographic scale (Bürger et al., 2020) and within specific fields (Perronne et al., 2014) or genotypes (Simons and Johnston, 2006). Intra-specific variability (ISV) can arise from genetic variability or phenotypic plasticity, primarily to enhance fitness, particularly in sessile organisms (Galloway, 2005).

Phenotypic plasticity could manifest when individuals show variability in their response to their environments within a generation, or when the parental (mostly maternal) environmental conditions affect the offspring phenotypes (Wadgymer et al., 2018). Failure to take into account spatial and temporal ISV may lead to unreliable or unsupported management decisions (Blank et al., 2023; Pichancourt et al., 2019).

Germination plays a crucial role in a plant's life cycle, as it defines the environmental conditions necessary for individual and population establishment (Donohue et al., 2010). Maternal plants are known to affect progeny germination, either through factors such as the seed coat properties (a direct effect of maternal tissue) or through maternal

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provisioning during seed development (Donohue, 2009). Understanding the effects of abiotic, biotic, and management factors on weed germination and emergence, and subsequently modeling these processes, are key steps for optimizing weed management in agricultural fields (Colbach et al., 2014; Grundy, 2003).

Temperature is the main abiotic condition controlling germination, particularly for spring-germinating species, when soil moisture is not a limiting factor in regions characterized by temperate or Mediterranean climates (Baskin and Baskin, 1988; Gardarin et al., 2010). Another important trait influencing germination is seed size, which can vary both between and within individuals and populations (Pélabon et al., 2021). Seed size affects germination parameters in various ways across multiple species (Baskin and Baskin, 2014 and references within). There are numerous reports suggesting that seed size and germination patterns of different species may be influenced by climatic gradients, although the findings remain inconclusive. For example, a decrease in both seed size and germination timing has been observed with increasing aridity (Christie et al., 2022) vs. a negative correlation between seed mass and mean annual precipitation (MAP, Cochran et al., 2016). Additionally, Dickman et al. (2019) recorded a reduction in the mean and variance of emergence time across populations of *Mimulus laciniatus* in response to drought, indicating an adaptive strategy for arid climate conditions. It is thus apparent that investigations of ISV in relation to germination or seed traits must take into consideration the appropriate geographic scale and the relevant climatic conditions (de Pedro et al., 2022).

The northern region of Israel provides an ideal location to explore variations in weed species populations. This region, spanning less than 100 km, is characterized by a marked climate gradient, transitioning from a semi-arid climate in the southeast to a Mediterranean climate in the north and west (Fig. 1). The variations in mean annual precipitation (predominantly rainfall, ranging from 250 to 700 mm) and spring mean temperatures (ranging from 13° to 24°C) present a unique range of conditions facilitating diverse crop planting seasons (Table 1). The soil disturbance resulting from conventional farming practices during the planting process thus occurs at different times during the germination

**Table 1**

Growing areas of the studied *A. albus* populations - geographical areas, long-term mean annual precipitation (MAP) and crop planting seasons.

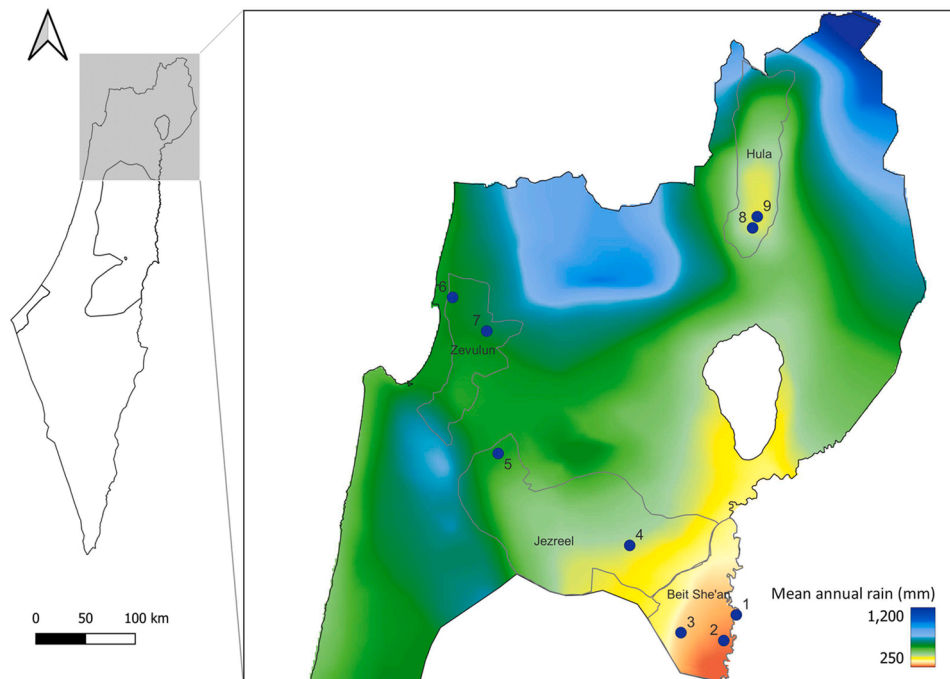
Location	Abbreviated name used in this study	Growing area	MAP (mm) <sup>a</sup>	Planting season <sup>b</sup>
Ma'oz Haim	Maoz	Beit She'an Valley	282	Late January to early March
Kfar Ruppim	Rupin	Beit She'an Valley	295	
Eden Farm	Eden	Beit She'an Valley	306	
Ein Harod Ihud	Harod	Eastern Jezreel Valley	423	
Newe Ya'ar	Newe	West Jezreel Valley	584	Late March up to mid-May
Gadash Re'em	Reem	Zevulun Valley	657	
Acco Farm	Acco	Zevulun Valley	603	
Hulata	Hulata	Hula Valley	420	
Ayelet Hashachar	Ayelet Hashachar	Hula Valley	473	

<sup>a</sup> Long-term MAP in the location where each population was collected, as recorded by meteorological stations of the Israel Meteorological Service (<https://ims.gov.il/he/ClimateAtlas>). The mean of the two nearest stations is shown for locations where there is no adjacent station.

<sup>b</sup> Planting season of agricultural spring crops (e.g., processing tomato).

periods of spring- and summer-germinating weeds in the various growing areas.

In this region, a particularly troublesome weed species is *Amaranthus albus*, an annual spring-germinating weed that competes with various crops, including processing tomatoes (Gafni et al., 2023). *A. albus* is a self-compatible monoecious species that is native to central North America but has spread to Europe, Africa, Asia, and Australia (Costea and Tardif, 2003). It is commonly found in disturbed, ruderal, and predominantly agricultural habitats (Assad et al., 2017). In Israel, it was



**Fig. 1.** Precipitation gradient in northern Israel. The map presents the study region and locations of nine *A. albus* populations (represented by blue circles) in their respective growing areas. These populations grow within an area characterized by a climate gradient from semi-arid in the southeast to Mediterranean in the west and north. The numbers denote the following *A. albus* populations: (1) Ma'oz Haim, (2) Kfar Ruppim, (3) Eden Farm, (4) Ein Harod Ihud, (5) Newe Ya'ar, (6) Acco Farm, (7) Gadash Reem, (8) Ayelet Hashachar, and (9) Hulata.

first reported in the late 19th century, and by the 1960s it had become widespread, particularly in environments influenced by anthropogenic activity, such as in spring- and summer irrigated fields (Zohary, 1966), as is the situation at present.

Numerous studies have reported variations in germination patterns among different *Amaranthus* species, highlighting the influence of maternal plant conditions. These include factors such as temperature (Kigel et al., 1977), water availability (Karimmojeni et al., 2014; Matzrafi et al., 2021), management practices (Brainard et al., 2007, 2006), solar radiation (Jha et al., 2010; Kigel et al., 1977), photoperiod, and the age of the maternal plants (Cristaudo et al., 2016; Faccini and Vitta, 2005; Kigel et al., 1979), as well as exposure to herbicides (Qi et al., 2018) and inherited resistance (Sibony and Rubin, 2003; Weaver and Thomas, 1986). Additionally, there are reports indicating that seed weights in *Amaranthus* species can be influenced by the age of the maternal plants (Cavers and Steel, 1984), soil water conditions during seed maturation (Matzrafi et al., 2021) and overall climate conditions, such as MAP (Schimpf, 1977). The influence of maternal conditions tends to diminish in the post-germination stages of different plant species (Bischoff and Müller-Schärer, 2010; Bürger et al., 2020). Despite various studies characterizing the growth and development of *Amaranthus* species (Guo and Al-Khatib, 2003; Horak and Loughin, 2000; Sellers et al., 2003; Spaunhorst et al., 2018), we found only one study that specifically examined the maternal effect on the growth of two *Amaranthus* species. In that study, Fenesi et al. (2014) reported that *A. retroflexus* and *A. albus* plants grown under drought stress or in high-resource environments influenced the response of their offspring, providing evidence – in the case of the *A. albus* – of maladaptive trans-generational plasticity.

Given that the studied populations referred to above were confined to irrigated agricultural settings, we question, here, how location-specific natural conditions (precipitation and temperature) affect seed traits, germination, and other phenological aspects. The purpose of this research is to characterize and understand the source of variability in germination response and early growth stages of *A. albus* populations obtained from agricultural fields spanning an environmental gradient. Based on this objective, we propose the following hypotheses:

1. Seed size will be affected by the amount of precipitation, leading to larger seeds in populations originating in locations with lower MAP. This adaptation allows for germination from deeper, more moisture-rich soil, and has also been reported in other *Amaranthus* species (Schimpf, 1977). Despite being collected from regularly irrigated fields, we assume that field-edge individuals, subjected to the local natural conditions, serve as a source for the within-field population.
2. Seed size will be determined primarily by phenotypic plasticity arising from the environmental conditions experienced by the mother plant during seed maturation.
3. The primary source of ISV in germination response is the phenotypic plasticity induced by environmental conditions experienced by the maternal plant.
4. Populations originating in late-planting growing areas will exhibit higher germination percentages under higher temperatures compared to populations from early-planting areas. This response is anticipated due to the soil disturbance occurring at a later stage during the germination period of *A. albus* for the late-planting populations.
5. Conditions experienced by the mother plants will not affect the post-germination growth of the progeny plants.

## 2. Materials and methods

### 2.1. Seed collection and plant material

Mature *A. albus* plants were collected in July 2017 from agricultural fields in four growing areas of processing tomatoes in Northern Israel:

the eastern Beit She'an Valley, the central Jezreel Valley, the western Zevulun Valley, and the north-eastern Hula Valley. A total of 21 fields were surveyed, and at least five mature plants were collected from each field. The plants (hence referred to as  $F_0$ ) were allowed to dry out, and the seeds (hence referred to as  $F_1$ ) were manually threshed and stored in complete darkness at 2 °C. At the start of the study, seeds from eight of these *A. albus* populations – i.e., seeds collected at particular locations in northern Israel, listed (with their abbreviations) in Table 1 – were randomly chosen to represent the different climate gradient and growing areas (Table 1, Fig. 1; also, see Supplementary Fig. S1 for the climate data for 2017). We had initially planned to use the Eden population (from the Beit She'an Valley, Table 1) in the experiments for evaluating the germination dynamics, but, due to a lack of sufficient material, the Eden population seeds were used only in the experiments conducted during the first year after harvest. Thus, we added a ninth population – the Rupin population – as an additional representative of the Beit She'an growing area.

In April 2019,  $F_1$  seeds from the eight populations (i.e., without the Eden population) were sown under net house conditions at the Newe Ya'ar Research Center (32°42'23.3"N 35°10'46.4"E), which is characterized by a Mediterranean climate. The aim was to produce  $F_2$  generation seeds unaffected by the climatic conditions of the source locations. The seeds were sown in 10 L pots filled with Newe Ya'ar soil (57% clay, 23% silt, and 20% sand). Upon emergence, seedlings were carefully removed until one plant was left per pot, with 10 pots representing each population. Temperature data were recorded using a Hobo data logger (Onset Ltd.), and the mean daily temperatures during the growth period were  $28.7 \pm 4.9$  °C. The plants were regularly irrigated and fertilized until they reached maturity and the seeds were ready for harvest. To prevent cross-pollination, each population was isolated within a polyethylene cage. The dried plants were threshed, and the  $F_2$  seeds were stored at 2 °C.

$F_1$  and  $F_2$  seeds were characterized (Section 2.2) and subjected to germination experiments under six constant temperature regimes (Section 2.3). Additionally,  $F_1$  seed germination dynamics were observed after different storage periods during the first year post-harvest. Fig. S2 in the supplementary material provides a scheme describing the work conducted throughout the study years.

### 2.2. Seed weight and morphology

Seed morphology and seed weight of all *A. albus* populations from both  $F_1$  and  $F_2$  generations were measured and compared. For each population in each generation, 18 batches of 50 seeds were weighed. Additionally, 800 seeds were randomly selected and scanned using an RH-2000 digital microscope (Hirox Co., Ltd). The area of each individual seed was calculated based on the 2D image, which facilitates the estimation of seed size.

### 2.3. Germination experiments

Germination experiments were conducted under controlled environmental conditions using growth chambers (Conviron Ltd., USA). For each population, 50 seeds were scattered on commercial potting mix (Tuff Substrates Ltd., Israel) in each of six Petri dishes. The seeds were watered and placed in complete darkness at 4 °C for two weeks. Thereafter, the Petri dishes were moved to trays assigned to specific temperature regimes, with a light-dark cycle of 15/9 h. Germinated seeds, defined as those with a radical length  $\geq 1$  mm, were recorded daily at the same time and removed from the dishes during the first two weeks and then every other day until the end of the experiments. To eliminate potential effects on germination of the position of the trays within the chambers, tray locations were randomly rearranged after each examination (Simons and Johnston, 2006). The experiments were terminated after 45 days or after a week without observed germination. Seed viability was assessed at the end of each temperature regime

experiment by applying a 0.002 M gibberellic acid (GA<sub>3</sub>) solution to ungerminated seeds in Petri dishes and transferring them to a growth chamber set at 30 °C (Brainard et al., 2007). The viability of all populations ranged from 80% to 100% for both generations (Supplementary Fig. S3).

The germination dynamics of the *A. albus* populations were assessed at 3, 6, 9, 12, and 36 months post-harvest. Initially, the germination dynamics of eight F<sub>1</sub> populations (Maoz, Eden, Hulata, Harod, Ayelet, Newe, Acco, and Reem) were followed during the first year at a constant temperature of 30 °C, known to be optimal for several *Amaranthus* species (Assad et al., 2017). At nine months post-harvest, the overall seed weight of each replication was measured to examine the influence of seed weight and MAP on germination rate. Additionally, the germination dynamics of eight populations for both the F<sub>1</sub> and F<sub>2</sub> generations were observed after one year of storage under six constant temperature regimes: 12, 18, 24, 27, 30, and 36 °C. As explained in Section 2.1, the Rupin population had replaced the Eden population (both from the eastern Beit-She'an growing region), and this population was thus examined only under 12, 18, 27, and 36 °C constant temperature regimes in the F<sub>1</sub> generation experiments.

#### 2.4. Common garden experiment

A common garden experiment was conducted in July 2020 at the Newe Ya'ar Research Center to assess early growth rate variability among *A. albus* populations and generations. Mean daily temperatures of 30.7 ± 1.3 °C were recorded using a Hobo data logger. Fifty seeds from each F<sub>1</sub> populations (excluding the Eden population) and their respective F<sub>2</sub> populations were planted in 10 L pots filled with local soil, with eight pots for each population and generation. The top layer of the soil (up to 5 cm depth) was sterilized (40 min at 121 °C) to prevent the emergence of local species of *Amaranthus*, as our *A. albus* populations started emerging at a depth of -3 cm (unpublished data). The first seedlings in each pot were tagged upon emergence and monitored daily until they reached the 10 true-leaf (TL) stage. To prevent competition, any seedlings emerging subsequently were carefully removed without damaging the monitored plants. Plants were irrigated as needed.

#### 2.5. Statistical analysis and germination model fitting

Statistical analysis and model fitting were performed using R 4.1.3 software (R Core Team, 2018). Data visualization utilized the 'ggplot2' package (Wickham, 2011). Weight differences between populations within each generation and differences between generations for each population were analyzed using a generalized linear model (GLM) with a Gamma distribution and a log link function. Post-hoc analyses were conducted using the 'emmeans' package (Lenth, 2022). As the normality assumption of seed size distribution was violated and GLM approaches were inadequate, a non-parametric statistical approach based on aligned rank transformation (ART, Wobbrock et al., 2011) was employed. Seed size comparisons across populations and generations were performed using the 'ARTool' package (Elkin et al., 2021). Variability of seed size estimates was quantified by manually computing the standard error of the mean, and the coefficient of variation (CV) was calculated using the 'EnvStats' package (Millard, 2013).

Germination data were analyzed using time-to-event models based on the approach described by Ritz et al. (2013). Parameter estimates for populations and generations were compared using a meta-analytic approach according to the methods outlined by Jensen et al., (2017, 2020). Due to the low germination percentages at 18 °C and the lack of germination at 12 °C, these temperature regimes were excluded from this analysis. To model cumulative germination over time, each replicate (Petri dish) from each temperature regime experiment was fitted to a log-logistic parametric model using the 'drc' package (Ritz et al., 2015):

$$F(t) = \frac{d}{1 + \exp(b(\log(t) - \log(t_{50})))} \quad (1)$$

where  $F(t)$  is the cumulative fraction of germinated seeds over time,  $d$  is the upper limit denoting the maximal germination potential,  $t_{50}$  represents the time taken for 50% germination (relative to  $d$ ), and  $b$  is the parameter related to the slope of the curve at time  $t_{50}$ . Petri dishes with less than 5% maximal germination were excluded. Parameter estimates from each replicate were incorporated into a hierarchical structured random-effects model according to the meta-analytic approach described by Jensen et al. (2020). Two linear mixed models were fitted. The first model included the interaction of population × time from harvest × model parameters for the F<sub>1</sub> population experiments at a constant temperature regime of 30 °C. The second model included the four-way interaction between generation, population, temperature regime, and model parameters, facilitating comparisons of germination patterns between different populations and generations under constant temperature regimes. The packages 'metafor' (Viechtbauer, 2010) and 'multcomp' (Hothorn et al., 2008) were utilized for this meta-analysis. In addition, R codes for visualization were employed (Jensen et al., 2020). Germination data at 18 °C were analyzed separately for each generation using the 'drctc' package (Onofri et al., 2022).

To assess the potential influence of the precipitation gradient on seed weight and germination rate, different statistical models were fitted and compared, including linear models and GLMs. Model performance was assessed using Akaike information criterion (AIC) scores and qq-plots. Furthermore, the mediating role of seed weight in the relationship between the precipitation amount and germination rate was investigated using the 'mediation' package (Tingley et al., 2014).

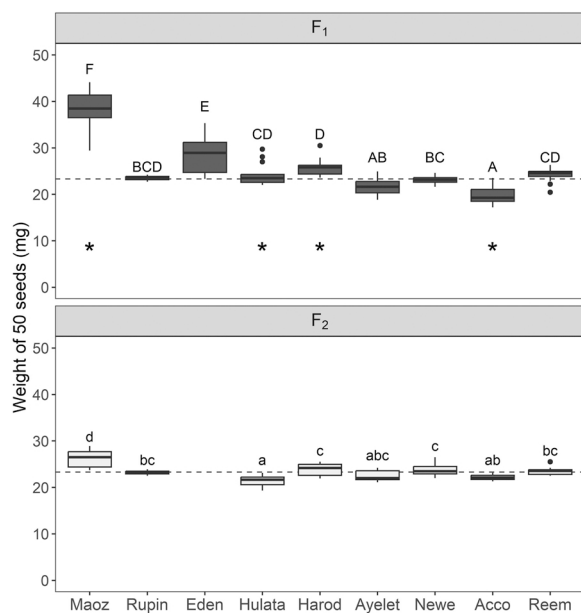
For the analysis of early growth in the common garden experiment, a linear mixed-effect model was employed to examine the time to appearance of true leaves. The model, fitted using the lmer() function from the 'lme4' package (Bates et al., 2015), included a three-way interaction of time, population, and generation, with the individual plant treated as a random effect. The intercept was constrained at  $y = 0$  to establish the baseline.

### 3. Results

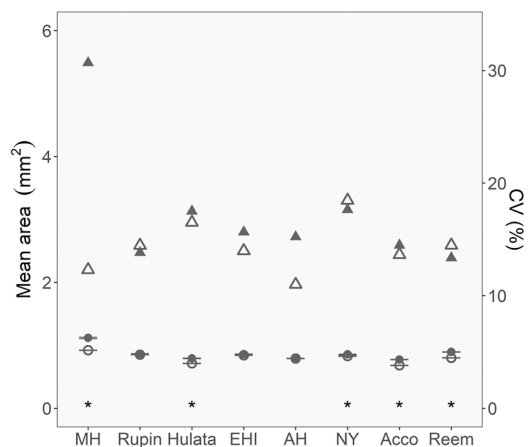
#### 3.1. Seed weight and morphology

There were significant differences in seed weight among the F<sub>1</sub> populations, with the eastern populations Maoz and Eden exhibiting the highest mean weights (38.5 and 28.6 mg per 50 seeds, respectively) and the western Acco population showing the lowest seed weight of 19.4 mg (Fig. 2). The overall mean weight of the F<sub>1</sub> populations was higher than that for the F<sub>2</sub> populations (25.5 vs. 23.4 mg;  $p < 0.001$ ). F<sub>2</sub> populations exhibited higher convergence around the mean with some differences in seed weight, although to a lesser extent than F<sub>1</sub> populations. The Maoz, Hulata, and Harod populations exhibited a decrease in seed weight from the F<sub>2</sub> to the F<sub>1</sub> generation. In contrast, the Acco population displayed an increase in mean seed weight in the F<sub>2</sub> generation (22.1 mg for F<sub>2</sub> compared to 19.8 mg for F<sub>1</sub>;  $p = 0.002$ ). Notably, for the Beit She'an region, the Rupin population had a lower seed weight vs. the other populations (vs. Maoz in both the F<sub>1</sub> and F<sub>2</sub> generations, and vs. Eden in the F<sub>1</sub> generation; Fig. 2).

Seed area was smaller in five out of the eight F<sub>2</sub> populations (Maoz, Hulata, Newe, Acco and Reem) compared to the F<sub>1</sub> generation. Seed area CV ranged from 12% to 27.4% for the F<sub>1</sub> populations and from 9.8% to 16.5% for the F<sub>2</sub> populations. The CV decreased for most progeny populations, with the Maoz population exhibiting the most significant reduction of 16% (Fig. 3).



**Fig. 2.** Comparison of seed weight in *A. albus* populations for  $F_1$  and  $F_2$  generations ordered according to their respective positions along the climate gradient. The top and bottom panels display the weights of batches of 50 seeds ( $n = 18$ ) from different  $F_1$  and  $F_2$  populations, respectively. Populations on the X-axis are arranged according to mean annual precipitation, from low (282 mm) to high (657 mm). Uppercase and lowercase letters denote significant differences in mean weight within the  $F_1$  and  $F_2$  generations, respectively. An asterisk (\*) indicates a significant difference between the two generations of a single population. All post-hoc comparisons were conducted using the Tukey HSD test ( $\alpha = 0.05$ ). The dashed lines in the two panels represent the overall mean seed weight of the  $F_2$  populations.



**Fig. 3.** Seed area variation in *A. albus* populations. The figure illustrates the mean area (circles) and coefficient of variation (CV, triangles) of *A. albus* seeds ( $n = 800$ ) in both the  $F_1$  and  $F_2$  generations across eight populations. The filled and open shapes indicate the  $F_1$  and  $F_2$  generations, respectively. An asterisk (\*) indicates a significant difference between the mean areas of the generations for a single population.

### 3.2. Post-harvest germination of the $F_1$ generation

None of the eight  $F_1$  *A. albus* populations germinated three months after harvest (before Rupin was included in the experiments). However, distinct germination patterns were observed across populations starting at six months post-harvest. Fig. 4 summarizes the estimated parameters for all the  $F_1$  populations in each run, while the complete germination data and fitted curves are presented in the [supplementary data](#)

(Supplementary Fig. S4). The Maoz and Eden populations (originating from the eastern region of the study area) consistently displayed rapid germination, with estimated maximal germination percentages ( $d$ ) of 74–85% and 84–88%, respectively. The western Reem population exhibited similar germination patterns with a maximal germination of 82–93%. The time to 50% germination ( $t_{50}$ ) did not exceed three days in all experiments for these three populations (Fig. 4). Contrarily, the remaining populations showed prolonged, less uniform germination patterns. The  $t_{50}$  values for the north-eastern Hulata population and the western Neue population ranged between 7 and 12 days and 9–12 days, respectively. However, three years post-harvest, all populations displayed a consistent pattern, with a  $t_{50}$  of < 3 days and similar estimated final germination of 85–92% (Fig. 4).

### 3.3. Effect of precipitation gradient on $F_1$ seed weight and germination rate

The precipitation gradient was found to be associated with both  $F_1$  seed weight and the time to 50% of maximal germination ( $t_{50}$ ) as estimated under a fixed temperature regime of 30 °C, nine months after harvest (Table 2, Fig. 5). All models were best represented by a GLM with a Gamma distribution and a log link-function. Our findings demonstrated a reduction in seed weight and a prolongation of  $t_{50}$ , indicating a delayed germination process, with an increase in MAP. Furthermore, we observed a negative effect of seed weight on  $t_{50}$ , with a coefficient estimate of  $-0.057$  ( $p < 0.001$ ). When considering both MAP and seed weight together as predictors of  $t_{50}$ , the coefficient estimate for precipitation was non-significant ( $p = 0.44$ ), while the effect of seed weight remained significant. This implies that seed weight fully mediated the impact of precipitation on germination rate. However, the mediation analysis revealed an insignificant mediation effect (Table 2).

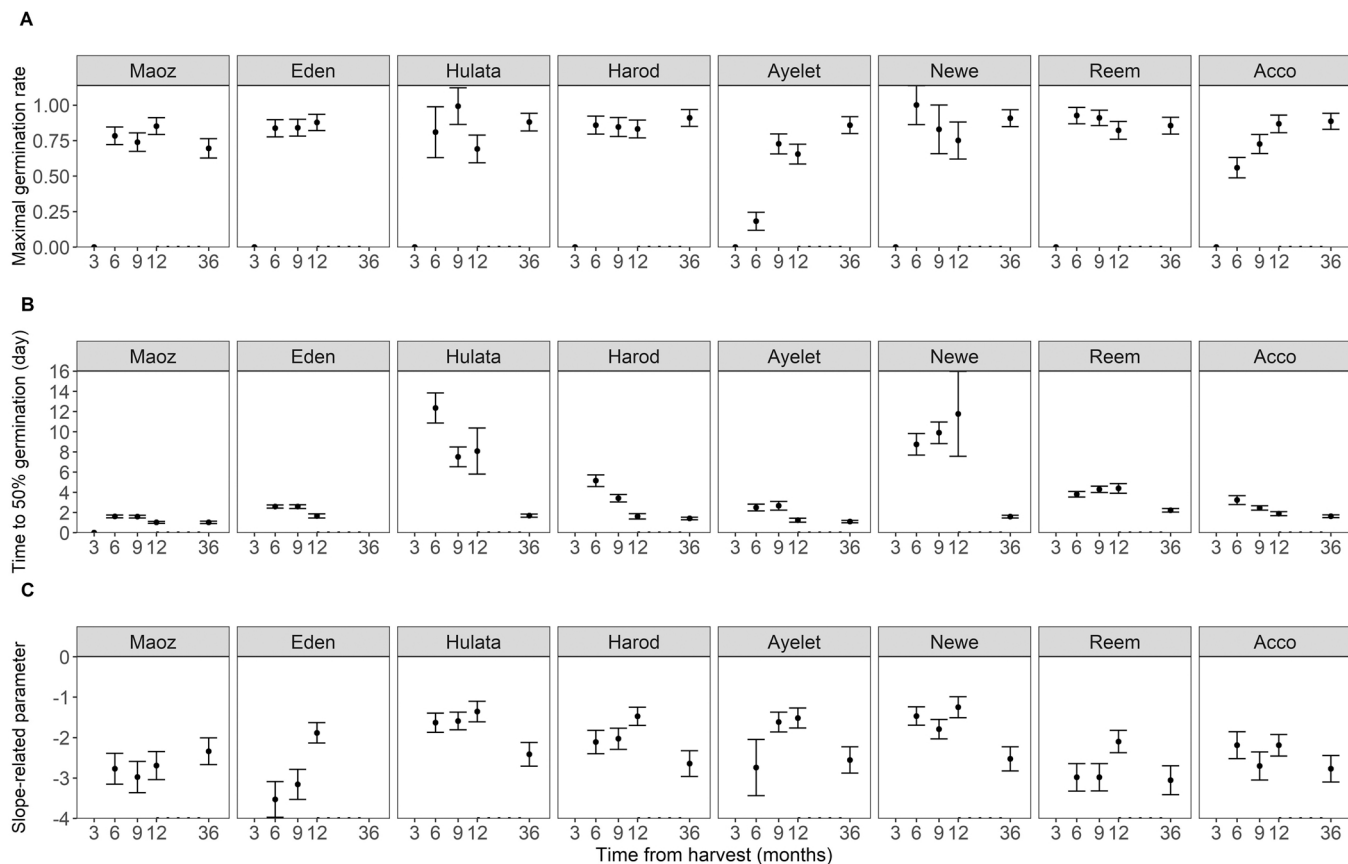
### 3.4. Effect of temperature and maternal conditions on germination

No germination occurred at a constant temperature of 12 °C for either the  $F_1$  or the  $F_2$  populations. At 18 °C, most populations exhibited relatively low germination percentages, with maximal germination ranging from 0.3% to 14% for the  $F_1$  generation and 5–26% for the  $F_2$  generation (Supplementary Fig. S5).

The  $F_2$  populations generally showed similar germination dynamics, characterized by rapid germination and higher maximum rates compared to their respective maternal populations at lower temperatures (24 °C and 27 °C) (Fig. 6; see Supplementary Table S1 for parameter estimates and Fig. S6 for the comparative analysis of  $d$  and  $t_{50}$  parameters across populations, generations and temperature regimes). Among the  $F_1$  populations, two distinct germination patterns were observed, which were particularly marked at a constant temperature of 30 °C. The Hulata and Neue populations were characterized by prolonged germination, with  $t_{50}$  values of  $8.0 \pm 1.2$  and  $11.5 \pm 2.2$  days, respectively, while all other populations exhibited a rapid and uniform germination, with estimated  $t_{50}$  of > 2 days. The western  $F_1$  Reem population displayed intermediate germination characteristics, with an estimated  $t_{50}$  of  $4.4 \pm 0.2$  days.

At 24 °C, the  $F_1$  populations from the late-planting growing areas (Ayelet, Neue, and Reem) exhibited a similarly low maximal germination of 10%. In contrast, the Maoz population from the early-planting growing area displayed a significantly higher maximal germination (60%, Fig. 6). As temperatures increased, maximal germination percentages across populations became more similar (Fig. S6). We note that comparing  $t_{50}$  estimates is complex, due to the relative nature of this parameter with respect to the upper asymptote. Most  $F_2$  populations at 24 °C had similar  $t_{50}$  values with similar  $d$  values ranging from 70% to 90%. At higher temperatures (30 °C and 36 °C), the  $t_{50}$  range was 0.9–3.0 days, despite  $d$  estimates that were close yet significantly different among  $F_2$  populations (Fig. S6).

Fig. 7 summarizes the contribution of temperature to the population



**Fig. 4.** Relationships between time after harvest and germination parameters predicted by the meta-analytical approach for *A. albus* F<sub>1</sub> populations. The figure presents the estimated parameters and confidence intervals (CI) of the log-logistic germination curves of the original F<sub>1</sub> population: (A) maximal germination, *d*; (B) time for 50% germination, *t*<sub>50</sub>; and (C) Slope-related parameter, *b*. To enhance visual clarity, the X-axis displaying the months from harvest has been rescaled.

**Table 2**

Statistical analysis using generalized linear models (GLMs) and structural equation modeling (SEM) to examine the relationships between mean annual precipitation (MAP), seed weight (SW), and time to 50% germination (*t*<sub>50</sub>) in *A. albus* seeds.

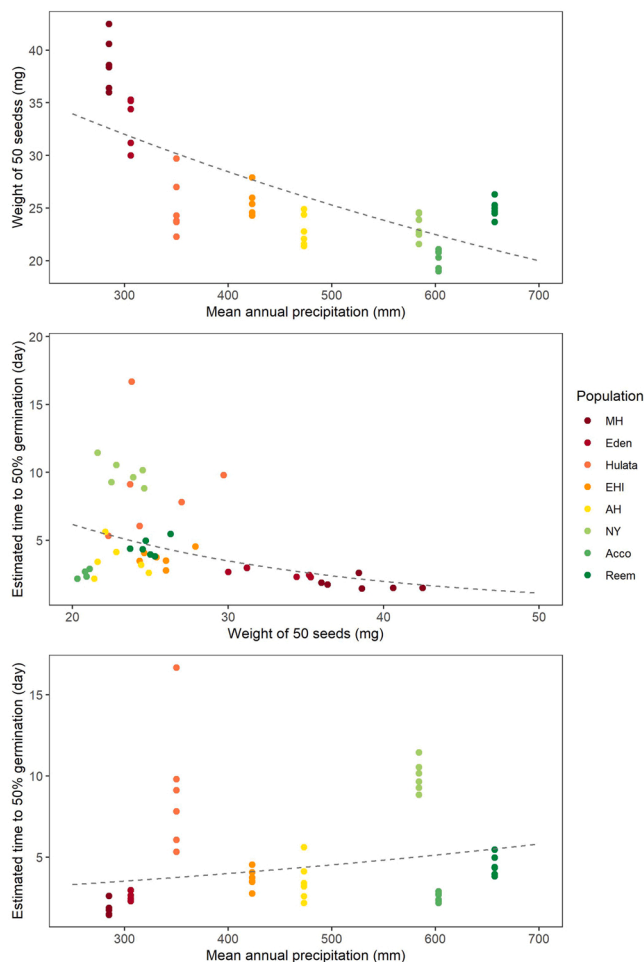
Model	Analysis	Coefficient	Estimate	Std. error	p-value		
<i>t</i> <sub>50</sub> ~ MAP	GLM	Intercept	0.882	0.215	< 0.001	** *	
		MAP	0.001	4.0e-04	0.006	**	
<i>t</i> <sub>50</sub> ~ SW	GLM	Intercept	2.962	0.228	< 0.001	** *	
		SW	-0.057	0.008	< 0.001	** *	
SW ~ MAP	GLM	Intercept	3.820	0.044	< 0.001	** *	
		MAP	-0.001	9.1e-05	< 0.001	** *	
<i>t</i> <sub>50</sub> ~ MAP + SW	GLM	Intercept	3.342	0.545	< 0.001	** *	
		MAP	4.2e-04	5.5e-04	0.444	NS	
		SW	-0.064	0.012	< 0.001	** *	
<i>t</i> <sub>50</sub> ~ MAP + SW	SEM	Effect	Estimate	LCI	UCI	p-value	
		ACME	0.087	-0.146	0.18	0.97	NS
		ADE	-0.001	-0.003	0.000	0.47	NS
		Total	0.086	-0.149	0.18	0.98	NS

Germination experiments were conducted nine months after harvest at a fixed temperature of 30 °C. The effect of seed weight, measured as the weight of 50 seeds in a single Petri dish (per observation), on *t*<sub>50</sub> was assessed through a time-to-event analysis. MAP was used as a proxy for the geographic origin of the F<sub>1</sub> populations. ACME = average causal mediation effect, ADE = average direct effect, LCI = lower confidence interval, UCI = upper confidence interval

germination fraction. F<sub>2</sub> seeds were more responsive to germination at lower temperatures (up to 24 °C). Conversely, F<sub>1</sub> populations, particularly from northern and western regions, displayed low germination percentages at 24 °C but increased percentages at 30 °C and 36 °C (Fig. 7). Only the eastern-most F<sub>1</sub> population (Maoz) exhibited a germination pattern similar to that of F<sub>2</sub> populations in terms of maximal germination (Fig. 7).

### 3.5. Common garden experiment

A linear regression model with the interaction of generation, population, and time was employed to analyze the growth rate over time. The results revealed a similar trend across populations and generations in the early stage, up to 10 true leaves (Fig. 8A). The growth rates ranged from 0.86 ± 0.02 to 0.95 ± 0.02 days to the appearance of a new true leaf (Fig. 8B), and no significant differences were observed between populations or generations.



**Fig. 5.** Relationships between mean annual precipitation, seed weight, and time to 50% germination ( $t_{50}$ ) in *A. albus* F<sub>1</sub> seeds. The top panel illustrates the relationship between seed weight and annual precipitation, the middle panel depicts the relationship between  $t_{50}$  and seed weight, and the bottom panel showcases the relationship between  $t_{50}$  and annual precipitation. Each dot represents a single observation, corresponding to a Petri dish with 50 seeds. The dashed lines in each panel represent the selected model, as described in Table 2. The  $t_{50}$  values were estimated through a time-to-event analysis following a germination experiment conducted nine months after harvest at a fixed temperature of 30 °C.

## 4. Discussion

### 4.1. Differences in mean seed weights and variability of seed traits

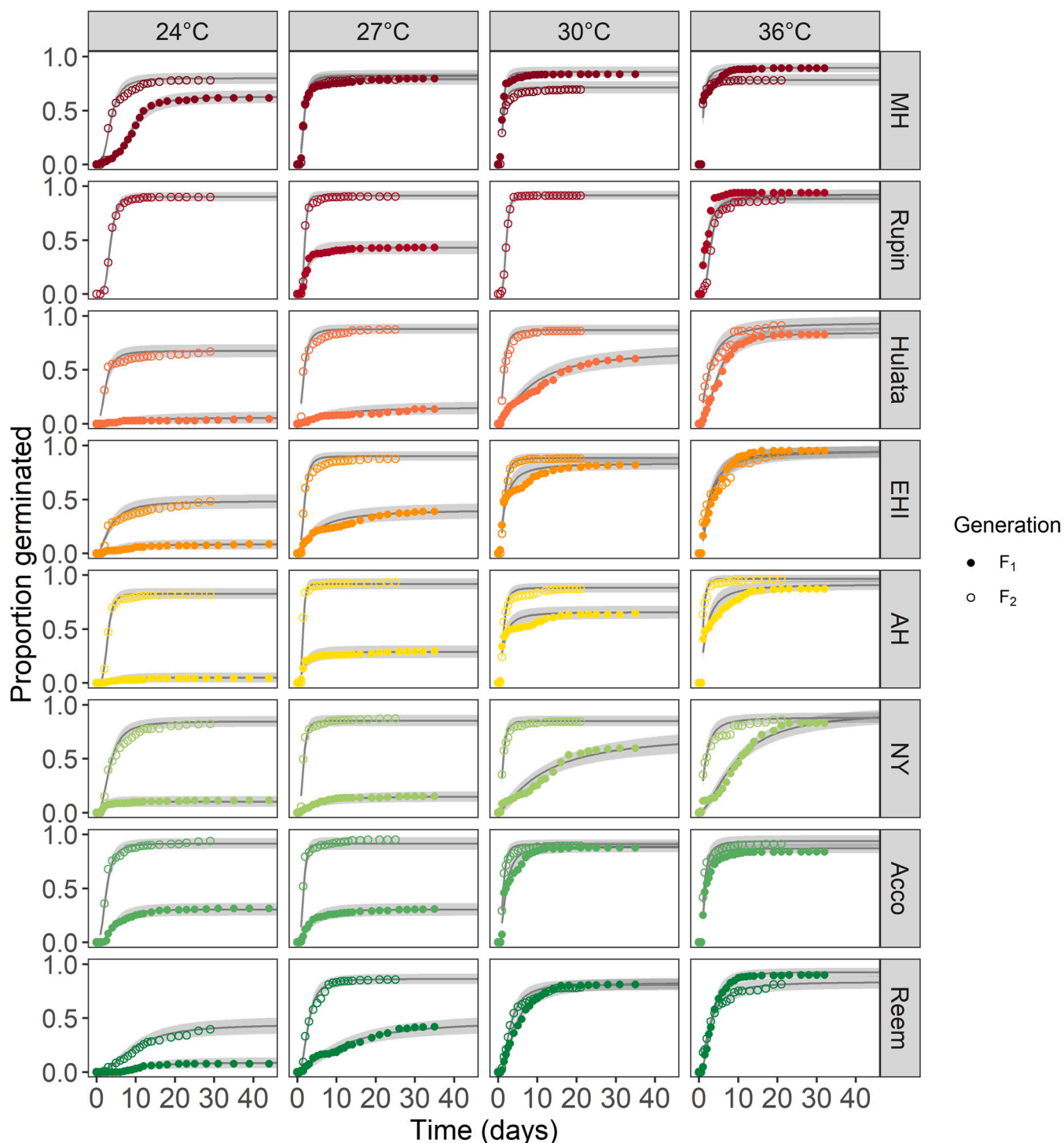
The effect of environmental gradients on seed characteristics and germination patterns has been widely reported, both between (Leishman et al., 2000; Metz et al., 2010) and within (Christie et al., 2022; Zhou et al., 2013) species. Since seed size is known to play a crucial role in determining the depth at which seeds can emerge, xeric habitats are expected to drive selection for larger seeds, enabling germination from deeper soil layers where water availability is less variable (Baskin and Baskin, 2014). Our research confirms our first hypothesis as we observed a significant increase in seed size as MAP decreases. This finding indicates a significant impact of natural habitat conditions on weed species, even those primarily associated with agricultural environments. These results align with the observations of Schimpf (1977) who reported a negative correlation between seed mass and MAP in populations of *A. retroflexus* originating from crop fields.

Increased ISV in seed traits can indicate a response to unpredictable and stressful environmental conditions and may be associated with

reduced heritability (Hoffmann and Merilä, 1999). Positive correlations were found between seed mass CV and both moisture and disturbance niche breadth (Kang et al., 2022), and also between seed size CV and higher variation in annual precipitation (Christie et al., 2022). Pélabon et al. (2021) suggested that variations in seed size can serve as a bet-hedging strategy to cope with unpredictable environments, particularly in terms of monthly rainfall fluctuations. Among the populations in this study, the Maoz and Eden populations obtained from the semi-arid Beit She'an Valley exhibited the highest variability in seed weight, with the Maoz population also displaying greater variability in seed area. These findings probably reflect the variability in environmental conditions in that area. However, in evaluating these findings, it is important to take into consideration the management practices in the different growing areas, especially the planting season for spring crops. Since seed size can be influenced by resource allocation from the mother plant and other indirect effects (Galloway, 2005), water availability is not the sole factor affecting seed size, and the length of the growing season will also play a role. In the eastern regions of the Beit She'an Valley and the eastern Jezreel Valley, the planting season for spring crops runs from late January to early March, coinciding with the onset of the germination season for *Amaranthus* species. In contrast, in the western and northern areas of the Zevulun, Hula, and western Jezreel Valleys, the planting season extends from late March to mid-May (Gafni et al., 2023). In all growing areas, the preparations for planting include the removal of all existing weed plants along with the early-season germinated fraction of *A. albus* in the seed bank, thus providing a 'clean baseline' for subsequent germination waves. Hence, in early-planting areas, indeterminate weeds can establish relatively early during the germination period and produce seeds continuously under changing environmental conditions. Here we note that although seeds were collected from the mother plants within a short time span (within two weeks), we cannot account for the duration of their maturation on the mother plant or the specific period in which they were produced. Previous studies have reported that both seed weight and variability can be affected by these factors (Cavers and Steel, 1984).

In contradiction to some of our initial expectations, not all populations examined in our study conformed to the observed trend. The Rupin population (from the Beit She'an Valley), originating from a relatively dry location (MAP of 295 mm), displayed greater similarity in terms of average and variation in seed size to the western populations. It is important to stress that the populations under investigation cannot be considered to be genetically closed due to the potential for human-mediated movement of seeds between locations. The shared use of mechanical equipment, such as trucks, spraying gear, cultivators, and combine harvesters, between growers from different areas may contribute to a gene flow between populations. Thus, it is possible that the Rupin population is a recent introduction. Another possibility could be that differences arise due to water availability in the agricultural setting, which may delay natural selection processes favoring larger seed sizes. Matzrafi et al. (2021) showed that *A. palmeri* plants grown under well-watered conditions produced smaller seeds compared with plants growing under water-deficit conditions.

Our study provides evidence for the high phenotypic plasticity of *A. albus* regarding seed size (both mass and area), thereby validating our second hypothesis. However, in the Maoz population, which originated from the driest location, the seed weight of the F<sub>2</sub> generations remained higher compared to the rest of the populations, even though they did exhibit a decrease. Most studies examining seed size along a climatic gradient have included origin populations growing under natural conditions. In contrast, our study focuses on *A. albus* populations obtained from agricultural fields that are regularly irrigated and fertilized. Nevertheless, we report the influence of the precipitation gradient on seed traits and germination patterns, providing strong evidence for the effect of field margins on the population within the field. These margins could provide weed species with the opportunity to adapt to the natural conditions in the habitat and facilitate establishment during seasons in



**Fig. 6.** Effect of temperature and maternal conditions on the cumulative germination of *A. albus* populations sampled across a climate gradient. The figure shows two generations, denoted by full and open dots representing F<sub>1</sub> and F<sub>2</sub> populations, respectively. The experiments were conducted one year post-harvest. The four constant temperature regimes are presented in the columns. Fitted log-logistic models, obtained through a meta-analytic approach, are depicted by gray lines and strips, accompanied by the corresponding confidence intervals. For detailed parameter estimates, see [Supplementary Table S1](#).

the crop cycle without a spring- or summer-irrigated crop.

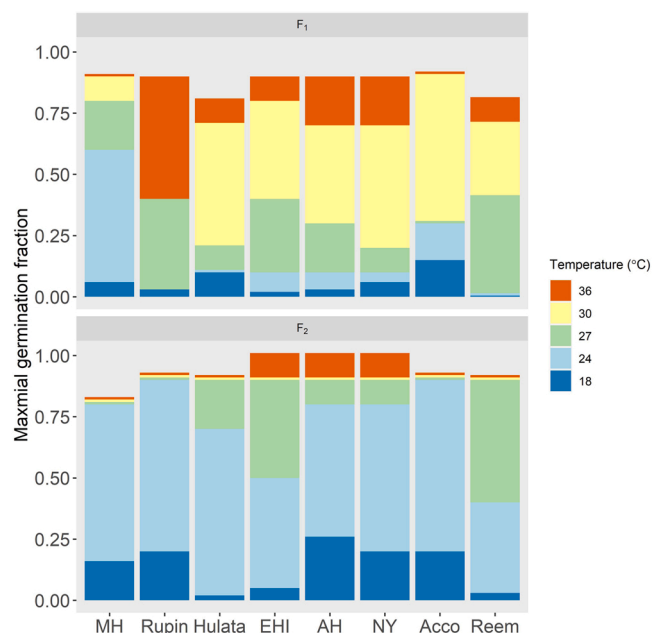
#### 4.2. Dormancy and germination patterns along the climate gradient

In our study, *A. albus* populations did not germinate after three months of dry storage. Primary dormancy, which plays a crucial role in protecting seedlings from unfavorable conditions, is commonly observed in *Amaranthus* species (Cristaudo et al., 2007; Khan et al.,

2022). Cristaudo et al. (2007) found that *A. albus* germination increased with longer storage times, peaking at 180 days post-harvest at temperatures of 20–40 °C.

After six months of dry storage, populations from early-planting regions, such as Maoz and Eden in the Beit She'an Valley, and Harod in the eastern Jezreel Valley, displayed a low level of dormancy. Conversely, populations from late-planting regions either exhibited a gradual release from dormancy (Ayelet and Acco) or a relatively high variability in





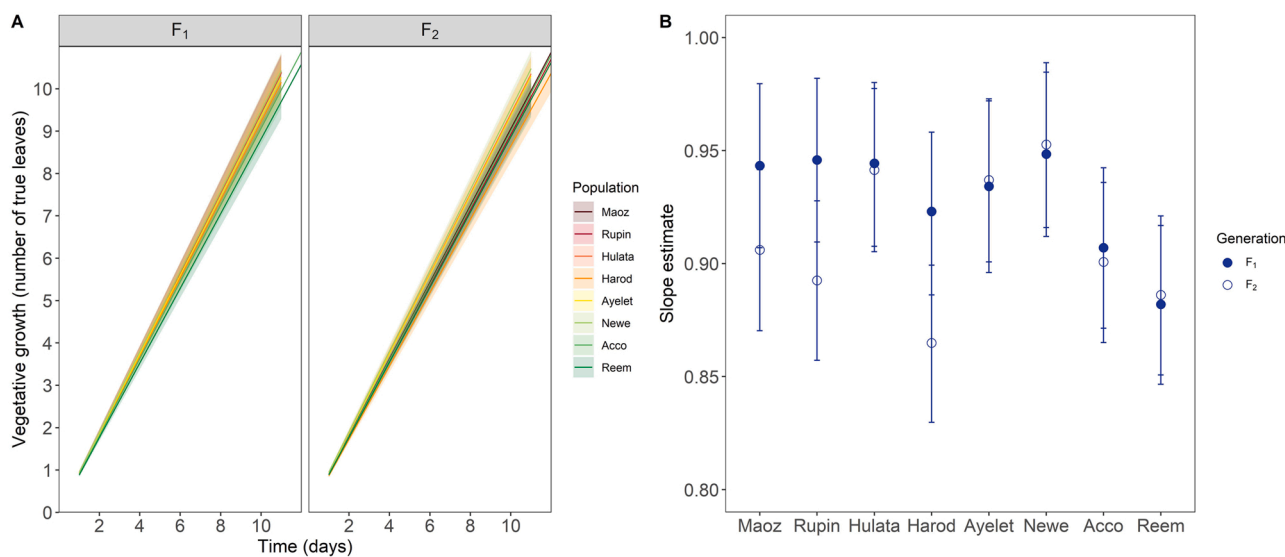
**Fig. 7.** Impact of constant temperature regimes on the maximum germination potential of *A. albus* populations (X axis). The upper and lower panels display the estimated additive germination fraction of non-dormant seeds at each temperature regime (indicated by different colors) for the F<sub>1</sub> and F<sub>2</sub> generations, respectively. The maximal germination estimates were derived through meta-analysis for the constant temperature regimes of 24, 27, 30 and 36 °C, while a time-to-event analysis with population as a grouping factor was conducted for 18 °C. However, for the MH population in the F<sub>2</sub> generation under 18 °C, the estimated *d* value ( $1.7 \pm 26.4$ ) was deemed unreasonable, and therefore, the observed mean final germination fraction (0.16) was used instead.

germination rate along with a prolonged germination time (Hulata and Newe). These findings suggest the effect of the management practices in the growing areas on the maternal plants and, subsequently, on the germination response of progeny seeds. In addition to supporting our third hypothesis, these novel findings provide a new perspective on the impact of management on germination. Kigel et al. (1977) showed that

the *A. retroflexus* seeds from plants grown in short-day conditions had higher germinability compared to those from long-day conditions. In the southeastern part of our study region, the planting season starts in late winter, resulting in seed maturation during the spring and early summer. In late-planting areas, early-emerging plants are removed from the field with plantings, and newly established plants produce seeds at the height of the summer under longer daylight conditions.

It is important to note that all populations, regardless of their origin, exhibited a lack of dormancy after three years. Similarly, Cristaudo et al. (2016) reported a complete dormancy break in *A. retroflexus* seeds at 400 days post-harvest, regardless of the environmental conditions or the age of the mother plants. These findings suggest that germination models should be interpreted with caution. Fields are regularly cultivated, burying new seeds and exposing older ones. Therefore, the germination observed in the fields probably represents seeds from past seasons. Thus, characterizing the germination patterns of different populations or of different generations of a single population is a necessary step for constructing a robust model.

Our findings regarding the effect of MAP and seed weight on the germination rate (*t*<sub>50</sub>) imply that the germination pattern is influenced by the climate gradient, with faster germination being observed in populations originating in drier locations. These results imply on the genetic adaptation of the origin populations to their environments, rather than phenotypic plasticity, thus undermining our third hypothesis. Albeit, caution is advised when interpreting them. When examining the effect of seed weight on *t*<sub>50</sub>, we observed that the trend was driven primarily by two populations, Maoz and Eden, both in the eastern Beit She’an Valley growing area. The variability in *t*<sub>50</sub> across populations at the lower range of seed weight was notably higher. Furthermore, we could not establish a mediating effect of seed weight on *t*<sub>50</sub>. While it may be argued that this discrepancy is due to misrepresentation along the gradients (MAP and seed weight), we propose an alternative explanation. The observed rapid germination to maximal rates in populations from early-planting growing areas might result from a high degree of intraspecific competition. Larger seeds not only exhibit better survival under drier conditions but also have an advantage under conditions of intense competition (Metz et al., 2010). Alongside the climate’s effect on seed size, reduced dormancy levels in the early-planting populations may favor the production of larger seeds.



**Fig. 8.** Early vegetative growth of *A. albus* populations. (A) Linear regression model depicting the effect of the interaction between time, generation, and population on the appearance of a new true leaf, along with the corresponding confidence intervals (CI). (B) Slope coefficients with estimated CIs, where filled and open circles represent the maternal and progeny generations of each population (X axis), respectively.

#### 4.3. Effects of temperature and maternal conditions on germination

The populations in our study did not germinate at a temperature of 12 °C, which is in keeping with previous research indicating a base temperature of 15.7 °C for germination of *A. albus* (Steinmaus et al., 2000) or no germination below 15 °C (Steckel et al., 2004). Our observations of high germination percentages at constant temperatures of 30 °C and 36 °C are consistent with the earlier findings on *A. albus* of Steinmaus et al. (2000), Steckel et al. (2004), and Cristaudo et al., (2014, 2007). The similar germination speed and proportion responses in the F<sub>2</sub> generation indicate that maternal-induced phenotypic plasticity appears to be the primary contributor to the variability observed in the studied populations, further strengthening our third hypothesis.

In this study, the only two populations that showed prolonged germination were from the late-planting regions, Hulata and Newe (with mean annual precipitations of 420 and 584 mm, respectively). We find it challenging to explain this unique pattern. However, since all populations were collected from arable fields, a possible explanation lies in the potential influence of herbicides. Qi et al. (2017) reported that the application of sub-lethal doses of atrazine or tribenuron-methyl, which are photosystem II (PSII) and acetolactate synthase (ALS) inhibitors, respectively, on *A. retroflexus* mother plants reduced seed germination percentages among their offspring. It should be noted that herbicides of the same groups are used by growers of processing tomatoes in Israel (Gafni et al., 2023), suggesting their application on the F<sub>0</sub> plants. However, the maximal germination of all populations in this study was unaffected, and under high temperatures, germination percentages reached near maximum potential. Simons and Johnston (2006) observed variability in the timing of seed germination within genotypes and proposed it as an adaptive strategy for coping with unpredictable environments. In general, agricultural environments are considered more unpredictable than the natural environment, and we cannot suggest a reason why the Newe and Hulata population's locations would be more unpredictable than the rest. Unraveling the factors responsible for this particular response exhibited by these two populations is beyond the scope of this study. We note, however, that this response is probably a result of maternal-induced plasticity, since it was not observed in the subsequent generation.

Our fourth hypothesis regarding the contribution of temperature to the germinating fraction of the populations was validated, providing further evidence to support our conclusion that the timing of crop planting influenced the germination response in different growing areas. The maternal Maoz population from the Beit She'an Valley demonstrated a 60% maximal germination at 24 °C. Unfortunately, we do not have the germination fractions for both generations of the other two populations from the Beit She'an Valley (Rupin and Eden) under all tested temperature conditions. However, we did test the Eden population at 24 °C (data not shown), and the estimated maximal germination was 22%, which is lower than Maoz but higher than all other populations. That having been said, the germination results for the Rupin population do not align with those for the other populations in its surroundings. The explanation for this finding may lie in its recent introduction, as mentioned earlier, or the possibility of local conditions overriding the maternal effect (Alba et al., 2016; Fernández-Pascual et al., 2013). It is evident that populations from the late-planting growing areas (Hulata, Ayelet, Reem, and Acco) reached high germination fractions only at 30 °C.

Our results emphasize the differences in the conditions experienced by the maternal and progeny populations during seed development. The F<sub>2</sub> populations exhibited high germination fractions at lower temperatures, similar to the pattern observed in the Maoz population. The progeny populations matured under well-watered conditions, which may have been similar to the conditions experienced by the Maoz population. It is worth noting that the year preceding the collection of the maternal populations was characterized as a very dry year (see Supplementary Fig. S1 for the 2017 climate records from meteorological

stations in different growing areas). Rainfall events during the early stages of the crop were mostly limited to the eastern regions (early plantings) and associated with low temperatures, suggesting higher water availability in the eastern fields.

#### 4.4. Early growth rate of *A. albus*

Our findings indicated that the initial growth rate of *A. albus* populations was not influenced by maternal conditions. These results align with our fifth hypothesis that maternal environmental conditions would not affect the post-germination growth of the progeny plants and are consistent with previous observations on *Amaranthus* (Bürger et al., 2020; Wulff, 1988) and other plant species (Bischoff and Müller-Schärer, 2010). It is expected that the current conditions experienced by the individual will determine its response (the moment it is able to respond), rather than past experience (Auge et al., 2017).

Our results also indicate a rapid growth rate of approximately one day for the addition of a new true leaf, which is a characteristic feature of the *Amaranthus* genus (Assad et al., 2017; Guo and Al-Khatib, 2003; Horak and Loughin, 2000). These findings hold significant agronomic implications, since the recommended stage for herbicide application in processing tomatoes is at the 2- to 4-true leaf stage, as advised by field extension services and agrochemical companies. This highlights the limited timeframe available for implementing chemical treatments (Horak and Loughin, 2000). However, it should be noted that we lack data for a growth comparison under semi-arid and early spring conditions, which would provide a more comprehensive understanding of the rapid growth rate of *Amaranthus* species.

Phenotypic plasticity of a functional trait is considered adaptive when it enhances the fitness of the invasive species, either by maintaining robustness in unfavorable environments or by exploiting favorable conditions, or both (Richards et al., 2006). Clearly, *A. albus* can be considered opportunistic, with the favorable conditions being the irrigated and fertilized agricultural setting, but this setting is also considered stressful and unpredictable due to high disturbance levels. While the species has a broad distribution in the study region, it does not appear to successfully invade natural environments. Nonetheless, we provide evidence of adaptation to local natural conditions, particularly in the semi-arid region.

## 5. Conclusions

This work sheds light on the ISV of *A. albus*, an important weed species in irrigated spring and summer crops. Along with other studies on both weeds and natural species describing ISV in germination parameters across a large geographic scale (Carhuanchó León et al., 2020; del Monte and Tarquis, 1997; Sugiyama, 2003; Tozzi et al., 2014), our findings enable broad conclusions to be drawn, potentially contributing to our understanding of how *A. albus* responds to local conditions and, by extension, providing insights into the impacts of climate change on weed species in these regions. These findings open up possibilities for incorporating other spring- and summer-germinating weed species into community ecology models once their variability is known (Siefert et al., 2015).

We report here for the first time the combined maternal effect of environmental and management factors (i.e., temperature and planting timing, respectively) on the germination response of *A. albus* populations to temperature. Early planting drove a trend to higher germination fractions under colder temperatures, while late planting favored germination at more favorable temperatures. Pichancourt et al. (2019) emphasized that "plasticity in demography limits the ability to upscale sensitivity results from site-year to higher scales." We claim that the highly plastic response to temperature observed in the F<sub>2</sub> populations indicates the potential for predicting *A. albus* germination patterns when the various components of the system are well understood.

One limitation of this study is the absence of additional

representation from the semi-arid growing area of the Beit She'an Valley. We recommend further investigation to encompass a wider range of ISV, including extreme climate conditions when possible.

The agricultural environment, being subject to constant disturbance, is significantly more complex than natural surroundings. This substantial variability has important implications for modeling, especially for models aimed at improving weed management. Our findings underscore the intricate interplay between natural processes and human-mediated factors in shaping the characteristics of weed populations and thereby indicate the need for location-specific tailored management instead of generalized management guidelines across regions.

### CRedit authorship contribution statement

**Roni Gafni:** Conceptualization, Methodology, Data curation, Software, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration. **Lior Blank:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Hanan Eizenberg:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

Data will be made available on request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eja.2023.127009](https://doi.org/10.1016/j.eja.2023.127009).

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