Planta (2020) 252:77
https://doi.org/10.1007/s00425-020-03491-2

ORIGINAL ARTICLE

Extreme drought alters progeny dispersal unit properties of winter wild oat (*Avena sterilis* L.)

Buzi Raviv[1] · Janardan Khadka[1] · Bupur Swetha[1] · Jeevan R. Singiri[1] · Rohith Grandhi[1] · Eliyahu Shapira[1] · Nurit Novoplansky[1] · Yitzchak Gutterman[1] · Ivan Galis[2] · Marcelo Sternberg[3] · Gideon Grafi[1]

Received: 7 June 2020 / Accepted: 4 October 2020 / Published online: 8 October 2020

Abstract

Main conclusion **The dead husk is a vital component of the dispersal unit whose biochemical properties can be modified following exposure to drought. This might affect seed performance and fate, soil properties and consequently plant biodiversity.**

Abstract We investigated the effects of extreme drought on the dispersal unit (DU) properties of winter wild oat (*Avena sterilis* L.) in the Mediterranean ecosystems focusing on a commonly ignored component of the DU, namely the dead floral bracts (husk). DUs were collected from a climate change experimental research station in the Judean Hills, Israel, simulating extreme drought and from two additional sites differing in the rainfall amounts. Our results showed that drought conditions significantly affected *A. sterilis* reproductive traits displaying reduced DUs and caryopses weights. The husk contributes profoundly to seed performance showing that germination from the intact DUs or the intact florets 1 was higher, faster and more homogenous compared to naked caryopses; no effect of drought on germination properties was observed. The husk stored hundreds of proteins that retain enzymatic activity and multiple metabolites including phytohormones. Changes in rainfall amounts affected the composition and levels of proteins and other metabolites accumulated in the husk, with a notable effect on abscisic acid (ABA). The husk of both control and drought plants released upon hydration substances that selectively inhibited other species seed germination as well as substances that promoted microbial growth. Our data showed that the dead husk represents a functional component of the DU that have been evolved to nurture the embryo and to ensure its success in its unique habitat. Furthermore, drought conditions can modify husk biochemical properties, which in turn might affect seed performance and fate, soil microbiota and soil fertility and consequently plant species diversity.

Keywords Allelopathy · Climate change · Dead organs enclosing embryos (DOEEs) · Dispersal unit · Drought · Husk properties · Maternal environment · Metabolomics · Phytohormones · Proteomics · Seed germination · Seed performance

Abbreviations

DOEE	Dead organs enclosing embryo
DU	Dispersal unit
JA	Jasmonic acid
LTER	Long-term ecological research
SA	Salicylic acid

Communicated by Dorothea Bartels.

Buzi Raviv, Janardan Khadka and Bupur Swetha contributed equally.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00425-020-03491-2) contains supplementary material, which is available to authorized users.

✉ Gideon Grafi
ggrafi@bgu.ac.il

[1] French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben Gurion, Israel

[2] Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama 710-0046, Japan

[3] School of Plant Sciences and Food Security, Wise Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel

Introduction

Recent findings highlighted previously unrecognized features in plant sexual reproduction whereby plants inherit to their offspring elaborated characteristics such as nutrition, growth factors and defense molecules, which are embedded within the dead organs enclosing embryos (DOEEs, reviewed in Raviv et al. 2018). The maternally derived DOEEs including seed coat, pericarps as well as floral bracts of the dispersal unit in grasses (e.g., glumes, lemmas and paleae, will be referred to as husk) store and release upon hydration hundreds of proteins (e.g., active hydrolytic enzymes, reactive oxygen species (ROS) detoxifying enzymes) and other substances that could contribute to seed persistence in the soil as well as to germination and seedling establishment (Raviv et al. 2017a, b, 2018; Godwin et al. 2017). The maternally derived DOEEs can act in multiple ways to ensure timely germination and to support growth and development of seedling. The pericarp and the seed coat as well as floral bracts and their accessories may function as mechanical barriers for water absorption (Cousens et al. 2010), gaseous exchange (Adkins et al. 2002; Xuejun et al. 2012) and radicle protrusion (Miyajima 1996) or as a storage for substances that are released upon hydration and either inhibit or promote seed germination (Kawaguchi et al. 1997; Sari et al. 2006) or affect microbial growth (Raviv et al. 2017b; Godwin et al. 2017; Prakash et al. 2018). In addition, they serve in seed dispersal and anchorage in the soil (Booth and Schuman 1983; Elbaum et al. 2007).

The effect of the husk/pericarp on seed germination is variable and species dependent. Several reports highlighted the negative effect of the husk on germination as husk/pericarp removal improved seed germination of *Aegilops kotschyi Boiss* (Wurzburger and Leshem 1969), *Zinnia elegans* (Ogawa and Iwabuchi 2001), *Aegilops cylindrica* (Fandrich and Mallory-Smith 2006), *Cladium jamaicense* (Webb et al. 2009), the crucifer *Lachnoloma lehmannii* (Mamut et al. 2014), *Sinapis alba* (Godwin et al. 2017) and *Atriplex centralasiatica* (Wang et al. 2019). On the other hand, multiple reports have shown that germination from the intact dispersal unit significantly improved germination and seedling establishment compared to naked seeds. This was described for *Atriplex polycarpa* (Sankary et al. 1972), *Eurotia lanata* (Booth and Schuman 1983), wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*, Raviv et al. 2017a), *Brachypodium hybridum* (El-Keblawy et al. 2019) and japonica rice (*Oryza sativa* L., Ueno and Miyoshi 2005). The pericarp of *Hedysarum scoparium* was shown to contribute to seed longevity and seedling establishment in arid environments (Hu et al. 2009).

As a result of global climate change, the frequency and severity of abiotic stresses are expected to increase leading to higher drought disaster-affected areas around the world (Li et al. 2009; IPCC 2018). In particular, climate change is predicted to affect the Mediterranean basin by increasing the incidence, duration and severity of droughts, together with prolonged dry spells and intensification of summer heat waves (Lionello et al. 2014; Spinoni et al. 2014). A continuous reduction in annual rainfall as well as shortening of the wet season have already been noted in the eastern Mediterranean region (Ziv et al. 2014). A recent study has shown that vulnerability of plant biomass production under different climate change scenarios in Israel increases with increasing aridity (Golodets et al. 2015). Additionally, changes in precipitation regime and extremes (increased drought or altered seasonality) are likely to have strong effects on Mediterranean ecosystems at different organizational levels, from ecosystem function level to individual plant traits levels (Alon and Sternberg 2019). Thus, most work related to plant behavior in response to climate change focused on phenological and physiological processes that eventually affect plant population dynamics and diversity. Nevertheless, the most important factor in determining dynamics and diversity of plants is the dispersal unit (Huang et al. 2016) whose properties (particularly the maternal component of the DU) have not been sufficiently studied under climate change-induced adverse environmental conditions. Considering that all DOEEs are maternally derived, it is likely that exposure of mother plants to stress conditions in the course of flowering and seed maturation might have an impact on the composition of substances accumulated within DOEEs. This in turn might have an impact on DOEE properties and their capabilities to support the seed during storage in the soil, germination and establishment.

With the aim of studying for the first time maternal growing effects of extreme drought and changes in rainfall patterns on the composition and level of substances stored within DOEEs, we selected natural populations of wild oat grass (*Avena sterilis*, Gramineae) growing in Israel. *Avena sterilis* has a wide distribution in Israel, from Mt. Hermon in the north to the Arava valley in the south and from the coastal plain in the west to the Jordan valley in the east (Feinbrun-Dothan and Danin 1998). It is an annual plant, from the Mediterranean-Irano-Turanian phytogeographical region that flowers during the spring time from March to May. The inflorescence of *A. sterilis* is a panicle of spikelets and at maturity the rachilla disarticulates below the lowermost floret only, leaving the conspicuous empty glumes on mother plants and thus generate the dispersal unit, which is composed of several florets (three–five), each may contain a single caryopsis enclosed by the dead lemma and palea. Each of the two basal florets (designated floret 1 and floret 2) has a dorsal awn while the upper florets are awnless. It is well documented that germination of caryopses within the

dispersal unit of *A. sterilis* is dependent on their position, whereby the first, basal floret germinates in the first year and the second and third florets in the second and the third years (Volis 2014).

We selected a long-term ecological research (LTER) site in the Judean Hills in Israel simulating extreme reduction in rainfall (drought conditions) from 675 mm (winter 2018–2019) to 180 mm (rainout shelters) as well as two sites along the precipitation gradients (727 mm and 155 mm) for studying the effect of rainfall amounts on the dispersal unit properties of *A. sterilis*. We employed various methodologies with the aim of exploring variation in DU (focusing on the husk) properties including germination assays, microbial growth assays, in-gel assays, as well as proteomics and metabolomics. Our data illuminated the significant effect of drought conditions on progeny DU of winter wild oat highlighting the elaborated function of the husk as a reservoir for beneficial proteins and substances that can affect seed performance and fate, soil properties and consequently plant biodiversity.

Materials and methods

Field sites and LTER design

Dispersal units (DUs) of *Avena sterilis* L. were collected on May 2019 from a climate change experimental research station simulating extreme drought and changes in rainfall distribution. This LTER Station is located at the Judean Hills, Israel (Matta LTER, N 31o 42′ E 35o 3′; 620 m a.s.l.). It receives an average of 540 mm annual precipitation (Israel Meteorological Service, IMS). Soil at the study site is *terra rossa*, developed on hard limestone and chalk. The vegetation consists of dwarf shrubs (dominated by *Sarcopoterium spinosum*) that cover approximately 20% of the area, and herbaceous annuals growing mostly in open areas and partly beneath shrubs. Annuals cover up to 80% of the open areas (~20% rocks and stones) and include approximately 80% of all species. Herbaceous plants (mostly annuals) account for 70% of the above-ground primary production (Sternberg et al. 2011). In general, the study area is characterized by high plant species richness, most of which is accounted by annual plants. *A. sterilis* is a common species growing at the site.

This study assessed an extreme drought scenario of 66% rainfall reduction following the Drought-Net protocols (https://drought-net.colostate.edu). Drought was achieved using rainout shelters that covered with greenhouse plastic sheets an area of 10 × 10 m. Rainout shelters reduced 66% of the long-term rainfall (540 mm). Changes in rainfall distribution were simulated by three irrigation events of 60 mm each (adding up to 180 mm), and monthly regularly distributed during the main rainfall season (December, January and February) using sprinklers located on the roof of the rainout shelters. Sprinklers used drizzle heads to reduce drops size and prevent any potential runoff generation. Drought plots were replicated five times. Rainout shelters were constructed during summer 2018, before the start of the rainfall season. Additionally, five plots of 10 × 10 m served as 'control' plots, and received natural rainfall and ambient conditions. Rainfall during the study period (October 2018–April 2019) was 675 mm. In addition, we collected DUs from two natural populations of *A. sterilis* growing on red soil (hamra) at Chafetz Haim site (CH, 31°47′ N 34°46′ E) characterized with 727 mm rainfall (CH727), and near Beer Sheva (BS, 31°18′ N 34°47′ E) on loessial soil with 155 mm rainfall (BS155).

Dispersal unit measurements, extraction of husk, in gel assays and statistical analysis

Measurement of the weight of the DUs and the caryopses were performed using an analytical balance. Husk was ground into fine particles and extracted (100 mg) in 1 mL of water or phosphate-buffered saline (PBS) by incubation overnight at 4 °C with gentle shaking. Samples were centrifuged at 16,000 *g* and the supernatant was collected and sterilized by passing through 0.22-µm PVDF filter unit (Millex-GV, Merck Millipore, Tullagreen, Carrigtwohill, IRL) and used in germination assays, bacterial growth and enzymatic activity assays. In-gel nuclease, chitinase and protease assays were performed essentially as described (Godwin et al. 2017). Statistical analysis (unpaired *t* test) was performed using the GraphPad QuickCalcs Web site: https://www.graphpad.com/quickcalcs/ttest1/?Format=C (accessed November 2019) or using the Microsoft Excel platform. All assays were repeated at least three times and representative results are shown.

Germination assays

Germination of *A. sterilis* DUs and separated caryopses were performed in pots containing red sandy soil in a net-house in Midreshet Ben Gurion during January (average temperature during the experiment period 15 °C day/7 °C night; $n = 12$). We daily inspected germination, that is, the emergence of the seedling from the soil and calculated the rate and the final percentage of germination. In addition, we performed growth chamber germination experiments of DUs versus caryopses under conditions of 16 °C day/8 °C night and photoperiod of 14/10 h light/dark. The number of DUs and caryopses used in these experiments are indicated in Fig. 2.

The effect of extracts obtained from caryopses and husks on germination of heterologous seeds (*Sinapis alba* L. and *Brassica juncea* L.) was performed in a Petri dish on a blot

paper and on red sandy soil supplemented with water or with husk extracts. Germination was performed in the dark at 22 °C, inspected daily and photographed. Seeds of *S. alba* were collected from natural populations growing near agricultural fields at northern Negev (31°, 26′, 42″ N; 34°, 39′, 37″ E). *B. juncea* seeds were purchased from the local market. All germination experiments were repeated at least three times and representative results are displayed.

Bacterial growth assay

The assay was performed essentially as described (Patton et al. 2006). Briefly, *Escherichia coli* (ATCC 10,978) were grown overnight on LB medium at 37 °C, the culture was diluted, transferred to 25% LB broth and grown at 37 °C to 0.03–0.05 optical density (OD_{595}; Epoch, Biotek, Winooski, VT, USA). To a 150 µL aliquot of the culture 50 µL of LB (control 1), PBS (control 2), ampicillin (final concentration 50 µg/mL), caryopsis extracts (50 µL) or 50 µL filtered (through 0.22 µm) husk extract (three replicates per treatment) was added in a flat-bottom 96-well microtiter plate. Plates were incubated in the dark using a spectrophotometer (Synergy 4, Biotek) and reads (OD_{595}) were taken in intervals of 30 min in a course of 12 h. The average OD for each blank replicate at a given time point was subtracted from the OD of each replicate treatment at the corresponding time point and standard errors were calculated for each treatment at every time point. Bacterial growth experiments were repeated three times using different husk extract preparations.

Proteome analysis

Proteome analysis of *A. sterilis* husk was performed by the proteomic services of The Smoler Protein Research Center at the Technion, Israel. Husk proteins extracted with PBS (three replicates each treatment) were digested with trypsin followed by separation and mass measurement on LC–MS/MS on LTQ-Orbitrap Protein identification and quantification were done using MaxQuant, using *Triticum* proteins from UniProt as a reference. Further bioinformatic analysis was performed as follows:

We started from protein-level LFQ-normalized intensities and used our in-house R script, with some more help from Excel and Partek Genomics Suite. The analysis included quality assessment of the raw and LFQ-normalized intensities and filtering out proteins marked as contaminant, reversed and only identified by site followed by Log_2 transformation of the LFQ intensities. Only proteins having at least two non-zero replicates in at least one of the treatment groups were retained. Differentially present (DP) proteins were identified after statistical testing with Limma. The statistical model tested the contrast between treatments. A protein was considered differentially present (DP) if it has passed the following cutoffs in at least eight of the ten imputed datasets: unadjusted P value < 0.05 and linear fold change < -2 or > 2 (where minus sign indicates down-regulation). Proteins having non-zero LFQ values in at least three samples (out of six, regardless of treatment group) were submitted to GO analyses using BiNGO. As a background for enrichment analysis, we used *Triticum* proteins downloaded from Uniprot.

Phytohormone analysis

Avena husks were chopped into small pieces and ground into fine powder in liquid nitrogen. Phytohormone extraction was done essentially as described in Fukumoto et al. (2013). Approximately 50 mg of each sample was taken and 1 mL of ethylacetate spiked with internal standards (IS) (D3-JA, D3-JA-Ile, D6-ABA, D4-SA) was added. Suspensions with ceramic beads were homogenized for 45 s in a ball mill FastPrep 24 (MP Biomedicals, Santa Ana, CA, USA) set to 5.0 intensity level. Samples were centrifuged at 16,000 *g*, 4 °C for 15 min, and cleared supernatants were transferred into new 2-mL microcentrifuge tubes. Pellets were re-extracted with 0.5 mL ethylacetate without IS, vortexed at room temperature for 5 min, and centrifuged as before. Supernatants from both extractions were combined and dried under vacuum. Before LC–MS analysis, dry extracts were dissolved in 300 µL 70% MeOH, and then diluted with 1.7 mL of 84 mM ammonium acetate buffer, pH 4.8, and passed through pre-conditioned solid phase extraction columns (3 mL size, Bond Elut-C18, 200 mg, Agilent Technologies, Santa Clara, CA, USA). After brief drying of columns with air, samples were eluted by hand using a syringe with 800 µL 85% methanol. Eluents were spun in a microcentrifuge and 10 µL cleared supernatant was applied on a triple quadrupole LC–MS/MS 6410 (Agilent Technologies), using previously reported instrument parameters and conditions (Fukumoto et al. 2013). Peak area of each endogenous phytohormone and internal standard was calculated using MassHunter Qualitative Analysis software (Agilent). Phytohormone concentrations were calculated by comparing peak areas of endogenous phytohormone to that of deuterium-labeled internal standards and expressed in per g dry-weight of sample.

Metabolite analysis

Extraction and quantification of primary metabolites were performed using GC–MS method essentially as described (Lisec et al. 2006). Briefly, plant tissues were ground in liquid nitrogen and the samples (40 mg) were extracted in 1.4 mL of 100% methanol with ribitol (12 µg) supplemented as an internal standard. The samples were homogenized for

2 min, incubated at 70 °C with shaking for 10 min. The samples were centrifuged at 4 °C for 10 min at 12,000 *g*. The supernatant was taken and vigorously mixed with 0.7 mL chloroform and 1.5 mL water. The phases were separated by centrifugation at 2000 *g* for 15 min and 150 µL of the upper polar phase was sampled and dried in vacuum concentrator at RT. The dried samples were sequentially derivatized with methoxyamine hydrochloride and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA). The derivatized samples were diluted (1/10) with dichloromethane and metabolites were analyzed with an Agilent 7890A-GC/240-MS instrument (Agilent Technologies). One microliter of each diluted sample was injected in split mode (1:10) into the injector port of the GC instrument held at 230 °C via an auto-sampler. Carrier gas (He) flow rate was set to 1 mL/min. Chromatography was performed on a HP-5MS capillary column (5% phenyl methyl silox, 30 m × 250 µm × 0.25 µm) (Agilent Technologies). The GC oven temperature was programmed at 60 °C for 3 min, followed by a 5 °C/min ramp to 300 °C and a final 5 min heating at 300 °C before returning to initial conditions. Mass spectra data were collected in full scan mode in mass range *m*/*z* 40–750. Metabolites were identified by comparing their fragmentation patterns with those of Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, MD, USA). Co-injection with authentic standards was performed to confirm tentative identifications. Quantification of metabolite concentration was done based on standard curves generated for each target compound and external standards.

Results

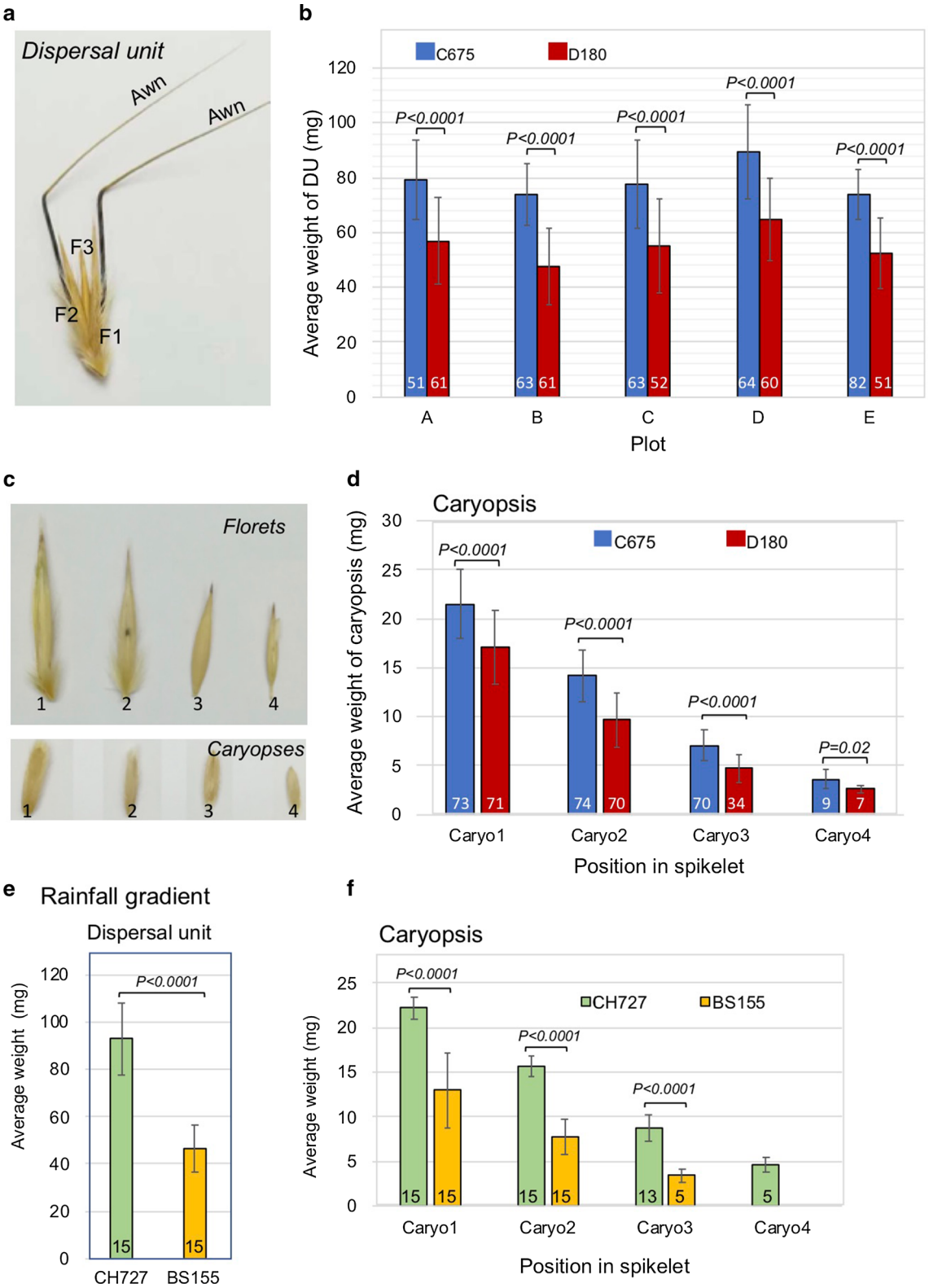
Effect of rainfall amounts on *A. sterilis* dispersal unit properties

Dispersal units (DUs) of *A. sterilis* (Fig. 1a) were collected on May 2019 from the experimental LTER site simulating extreme reduction in rainfall from 675 mm (Control, C675 plots) to 180 mm (rainout shelters; drought, D180 plots) as well as two sites along the precipitation gradients displaying 727 mm (Chafetz Haim, CH727) and 155 mm (Beer Sheva, BS155). The average weight of the DUs collected from the D180 plots was significantly reduced compared to control plots (Fig. 1b). Since each DU contain multiple florets, each can give rise to production of a caryopsis (Fig. 1c), we also measured the effect of drought on the weight of each caryopsis within the DU showing a significant reduction in caryopsis weight at each position of the DU (Fig. 1d). About half of the DU collected from D180 plot lacked the caryopsis at position 3 and in both treatments, the presence of caryopses at position 4 was dramatically reduced. Likewise, the weight of DUs collected from CH727 was significantly higher than those collected from BS155 (Fig. 1e). The weight of caryopses collected from CH727 site was significantly higher than those collected from BS155 displaying 1.7-, 2-, and 2.5-fold for caryopsis 1, 2 and 3, respectively (Fig. 1f). Thus, the results showed that reduction in precipitation will have a significant effect on reproductive traits in natural populations of *A. sterilis*.

The effect of rainfall amounts on progeny seed germination

Next, we examined the effect of rainfall on germination capacity of progeny seeds comparing between *Avena* DUs derived from C675 and D180 plots. Accordingly, DUs were dissected into floret 1 and caryopsis 1 (floret 2 is essentially dormant, Volis 2014) and sown in pots containing red sandy soil during the winter (January, average temp day/night 15/7 °C). Germination, that is emergence of the coleoptile, was evident 10 days after sowing (DAS) showing that floret 1 from both control and drought plots were germinated at high percentages (100% and 92%, respectively). A reduction in germination was observed for the naked caryopses derived from floret 1 collected from C675 (58% germination) and D180 (42% germination) plots (Fig. 2a). This demonstrates the beneficial impact that the husk (lemma and palea) have on germination and seedling emergence. Moreover, careful inspection of the rate of germination revealed (Fig. 2a) that germination from the intact DU is essentially homogenous compared to the naked caryopsis; high emergence of F1 seedlings were observed at 10 DAS for C675 (100%) and D180 (83%). At the same time (10 DAS), germination of naked caryopses 1 was lagging behind the florets, displaying 42% and 8.3% germination for caryopses derived from C675 and D180 plots, respectively. We repeated this experiment (Fig. 2b, c) using a controlled growth chamber under similar growth conditions (16 °C/8 °C day/night; 14/10 h photoperiod) and found similar results, that is, germination from the DU is higher, faster and more homogenous than from the naked caryopses. Yet we could not detect significant differences in final emergence (Fig. 2d) or in emergence index (Fig. 2e; Kader 2005) between C675 and D180 suggesting that the beneficial effect of the husk is preserved in DUs regardless of maternal growth conditions.

Similar promoting effect of the husk on germination rate was observed with wild emmer wheat (*Triticum turgidum ssp. dicoccoides*) collected at Tabgha near the sea of Galilee, whose DU (the spikelet) contains two florets, upper and lower. Commonly, the upper floret that contains larger grain than the lower floret is promptly germinated, while the lower floret often remains dormant in the first year (Nave et al. 2016). We analyzed germination properties of the intact DU comparing it to the dissected upper and lower florets and the naked caryopsis derived from them. Results showed



(Supplementary Fig. S1) that the final germination percentage of the intact DU and the upper floret was the highest displaying 87% and 80% germination, respectively; the naked caryopsis derived from the upper floret had a slightly lower germination (67%). We observed 33% and 7% germination for the lower floret and its naked caryopsis, respectively. Most noticeable, however, is the rate of emergence from soil, which was faster and more homogenous for the DU and the

◂**Fig. 1** Significant decline in seed weight of natural population of *A. sterilis* following reduction in availability of water. **a** The DU of *A. sterilis* composed of florets (F1–F3 are visible). Awns on the lemmas of the two lower florets (F1 and F2) are indicated. **b** Average weight of the DU in control uncovered plots (C675) and rainout shelter plots (D180). **c** Florets separated from the DU (upper panel) and their caryopses (lower panel). **d** Average weight of caryopsis at different floret location within the DU. **e**, **f** Analysis of natural populations of *A. sterilis* along precipitation gradient at Chafetz-Haim (CH727) and Beer Sheva (BS155). **e** Average weight of DU, **f** average weight of caryopsis (caryo1–caryo4) at different floret location. Numbers at the bottom of each column indicate the number of DUs or caryopses analyzed. Vertical bars represent the standard deviation. Note, DU weight was taken without owns. Differences are statistically significant as determined by two-tailed *P* value using unpaired t test with Graphpad software

upper floret and more diverse for naked upper caryopsis and the lower floret (Figs. S1a, S1b).

The effect of husks on seed germination of heterologous species

The finding that germination from the intact DU or the floret is largely improved over germination from the naked caryopsis prompted us to investigate for the presence of potential germination promoting substances in the husk. We performed a preliminary experiment to examine the effect of husk and caryopsis aqueous extracts of *A. sterilis* DU (C675 plot) on germination of *Sinapis alba* seeds. Interestingly, extracts obtained from the husk of floret 1 (HuskF1) and floret 2 (HuskF2) significantly inhibited *Sinapis* seed germination. The effect of extracts derived from caryopsis 1 (caryo1) and caryopsis 2 (caryo2) were essentially indistinguishable from that of water (cont/H_2O) (Supplementary Fig. S2).

We investigated the effect of maternal environment on husk germination inhibitory properties. To this end, we analyzed the effect of husk extracts from C675 and D180 DUs on seed germination of *S. alba* and *Brassica juncea* in comparison to germination in water. Interestingly, husk extracts of both C675 and D180 displayed selective germination inhibitory effect, that is, husk extracts derived from C675 and D180 inhibited seed germination of *S. alba* but not of *B. juncea* (Fig. 3a). Notably, *S. alba* seed germination could be partially recovered within 48 h following washing and incubation in water (Fig. 3b). Thus, we could not detect a clear effect of maternal environment on husk germination inhibitory properties and the effect of D180 was indistinguishable from that of C675.

Similar results were obtained when germination experiments were performed on red sandy soil. Germination of *B. juncea* was not affected by husk extract derived from C675 and D180, while germination of *S. alba* was strongly inhibited (Supplementary Fig. S3). *Sinapis alba* seeds were only marginally recovered after 48 h following washing and incubation on red sandy soil irrigated with water (Fig. S3).

Hundreds of proteins are released from husks upon hydration: proteome analysis

We investigated the protein composition in the husk of *A. sterilis* collected from the LTER station comparing C675 with D180. Husk proteins extracted from *A. sterilis* DUs (C675 and D180) were subjected to proteome analysis using LC–MS/MS on LTQ-Orbitrap followed by identification and quantification by MaxQuant, using *Triticum* proteins from UniProt as a reference (Table S1). Implementing the cutoffs (No. peptides > 1) we identified 455 proteins that were released from the husk of *A. sterilis* (Table S2). Among these proteins the level of 162 proteins was changed by at least two-fold.

Functional categorization for biological process revealed that among the proteins recognized in this category, 127 proteins are related to oxidation–reduction process and 54 proteins are involved in response to stimulus (Fig. 4a). Many of the categories displayed over twofold enrichment including protein folding and response to various stimuli (Fig. 4b). Molecular function analysis highlighted several groups of proteins including hydrolases (121 proteins), oxireductases (114 proteins) and peptidases (35 proteins) (Fig. 4c). Multiple hydrolytic enzymes listed in the proteome data (Table S3) include nuclease S1, a homolog of the *Arabidopsis* BFN2, BIFUNCTIONAL NUCLEASE 2 (ENDO2 encoded by At1g68290) involved in leaf senescence and cell death (Granot et al. 2015). Also, we identified chitinases, which might be involved in chitin catabolism and in response to biotic stresses (reviewed in Grover 2012), as well as multiple peptidases (Table S3). Other protein groups include heat shock proteins (HSP16.9; HSP101), reactive oxygen species (ROS) detoxifying enzymes (superoxide dismutases, catalase and peroxidases) as well as cell wall modification enzymes (pectinesterase and β-galactosidase) (Table S3). Comparing the proteins extracted from the husk derived from C675 and D180, we identified 36 differentially present (DP) proteins that passed the cutoff (P value < 0.05) (Table 1). Among them, 5 proteins were downregulated and 31 proteins were upregulated in D180 husks, many of which are proteins involved in response to stress.

Analysis of hydrolase activities

The proteome data highlighted certain protein groups released from *Avena* husk including nucleases, chitinases and proteases (Table S3). To examine the effect of rainfall amounts on their enzymatic activities we performed in gel assay comparing hydrolytic activities released from the

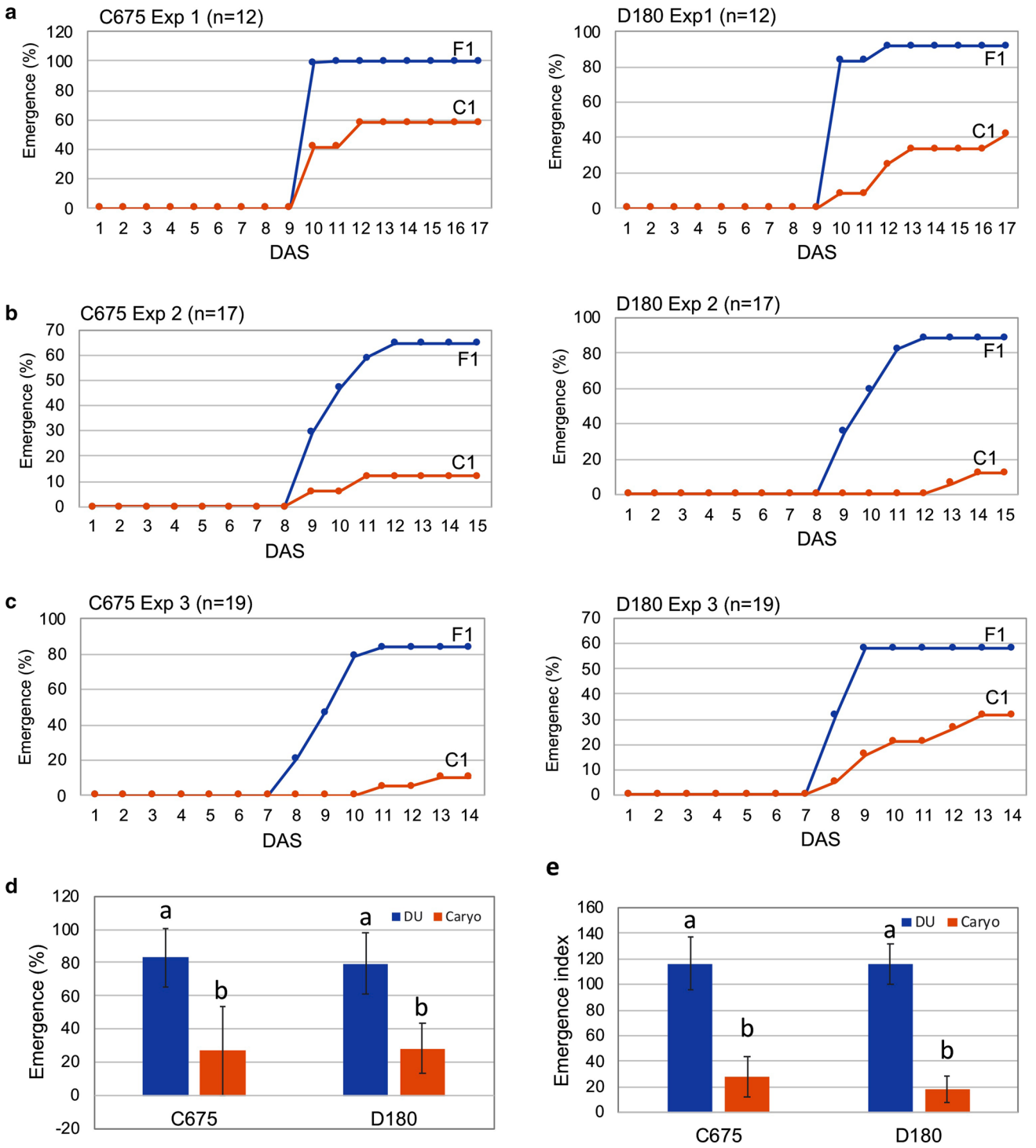


Fig. 2 Emergence rate of *A. sterilis* seedlings arise from the intact florets or naked caryopses derived from plants grown at C675 and D180 plots. **a**–**c** Repeated germination/emergence experiments. Germination was performed on red sandy soil at a net house during January 2020 (Av. day/night temp 15/7 °C) (**a**) or in a growth chamber set to 16/8 °C and photoperiod of 14/10 h light/dark (**b**, **c**). The rate of emergence is given as cumulative percentage of emerging seedlings at a given day after sowing (DAS). *F1* floret 1, *C1* naked caryopsis of F1. **d** Final percentage of germination/emergence. **e** Germination (i.e., emergence) index (GI, Kader 2005) of the various components of the DU. Different letters indicate statistically significant differences between treatments ($P < 0.05$; Student's unpaired *t* test)

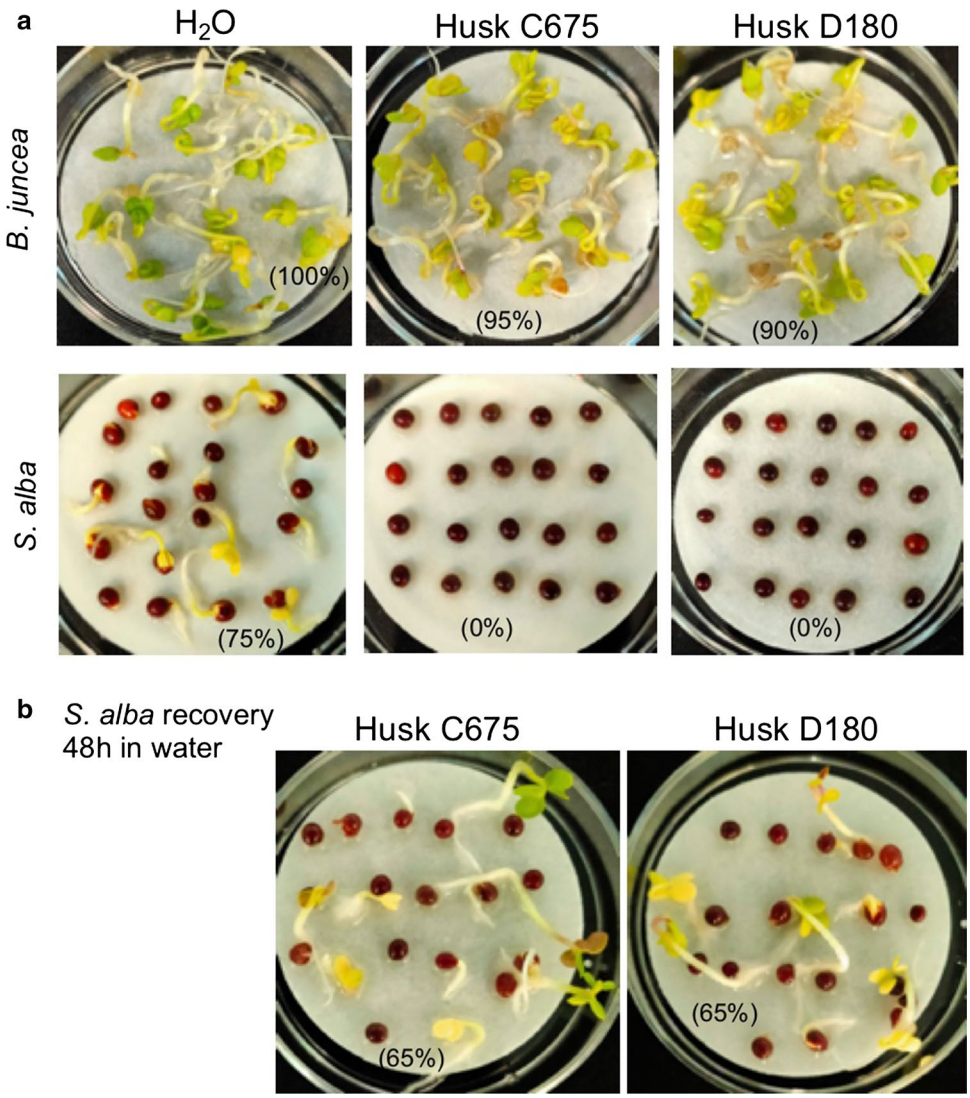


Fig. 3 The husk of *A. sterilis* DU possesses allelopathic substances that specifically inhibit seed germination of *S. alba* but not of *B. juncea*. **a** The husk of *Avena* DUs collected from plants grown at C675 or D180 plots were extracted with water and applied for germination of *S. alba* and *B. juncea* seeds in comparison to water (H_2O). Germination capacity was monitored after 96 h. **b** Germination recovery of *S. alba* seeds preincubated for 96 h in husk extracts following washing and incubation for 48 h in water. Final percentage of germination is given in brackets

husk obtained from plants grown at high (C675, CH727) and low (D180, BS155) rainfall conditions. The analysis of hydrolase activities (Fig. 5a, Exp1 and Exp2) showed that reducing water availability both in the LETR station (D180) or in the site near Beer Sheva (BS155) had a notable effect on hydrolase activities released from the husk. Accordingly, we found changes in the pattern and activity of nucleases and proteases under drought conditions but no notable effect on chitinase activity (Fig. 5a). The proteome data also listed HSP proteins in the husks (Table 1 and Supplemental Table S3) including HSP70 and the small HSP16.9, which was elevated 25-fold in D180. The proteome data were confirmed by immunoblotting using antibodies to HSP70 and HSP17.6 showing a significant increase in level of small HSPs in D180 husks (Fig. 5b).

Effect of precipitation on the accumulation of phytohormones and primary metabolites in the husk of *A. sterilis*

Husk derived from *A. sterilis* plants grown at the LTER plots C675 and D180 or from natural population at Chafetz Haim (CH727) and Beer Sheva (B155) were subjected to phytohormone analysis using liquid chromatography–mass spectrometry (LCMS). This analysis revealed that the husk stores several phytohormones and growth regulators including ABA, SA and JA. Most notable is an increase in ABA level in husk derived from plants experiencing drought, namely D180 and BS155 (Fig. 6).

SA and JA and its conjugated derivative JA-Isoleucine (JA-Ile) were accumulated in the husk with no notable

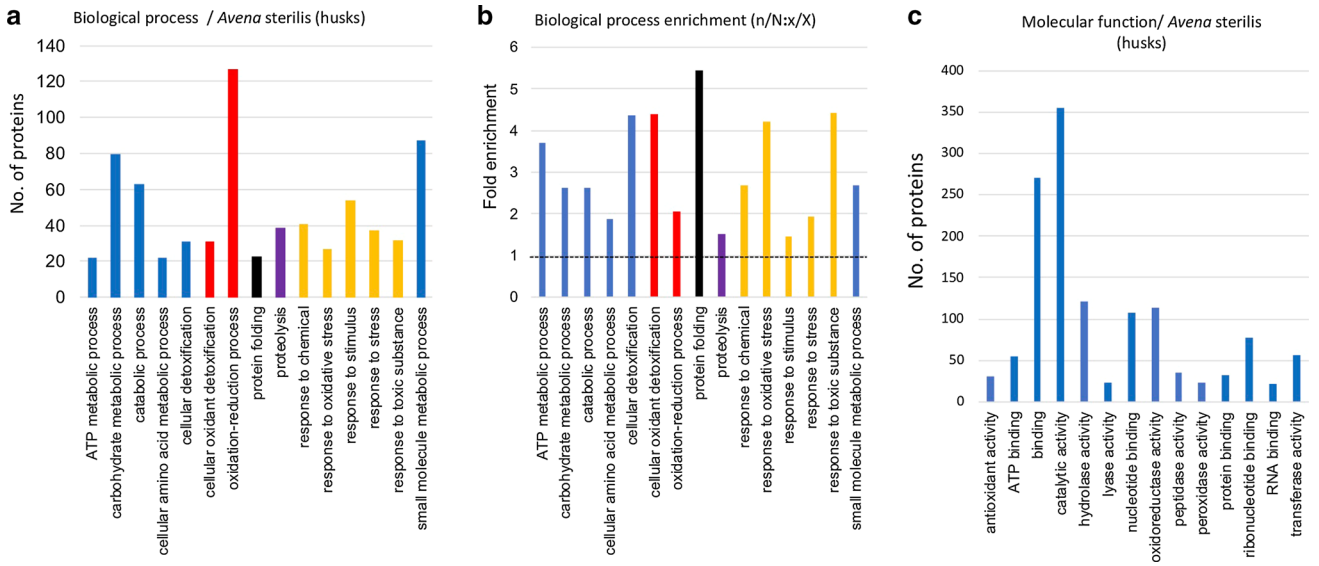


Fig. 4 Gene ontology (GO) categorization of proteins released from the husk of *A. sterilis* DU. **a** Functional categorization for biological process. GO categories involved in response to various stimuli, protein folding, proteolysis and oxidation–reduction process are highlighted yellow, black, purple and red, respectively. **b** Enrichment of GO categories for biological process. *n* number of husk's proteins identified in a given category. *N* Total number of husk's proteins. *x* number of gene-encoding proteins annotated to a given category in the *Avena* genome. *X* Total number of gene-encoding proteins in the *Avena* genome. **c** Categorization for molecular function. Functional categorization and GO term enrichment were performed using BiNGO (Maere et al. 2005)

effect of precipitation on their level, except in plot A where SA showed elevated level (sixfold).

GCMS analysis of metabolites showed the accumulation of free amino acids in husk of *A. sterilis*. Interestingly, many of the amino acids examined including serine, alanine, leucine and proline as well as the non-protein amino acid γ-aminobutyric acid (GABA) tend to accumulate to high levels in floral bracts derived from natural population of *Avena* experiencing drought (BS155) (Supplementary Fig. S3).

The metabolic analysis also revealed the presence of multiple sugars including fructose, glucose, galactose and sucrose in husk of *A. sterilis* with a notable elevation in floral bracts derived from BS155 (Supplementary Fig. S4).

Substances released from the husk of *A. sterilis* promote bacterial growth

Germination could be a vulnerable stage in the plant life cycle, as the embryo germinates into a potentially hostile environment populated with multiple types of microorganisms, some of which are pathogenic. Previously, we have shown that substances released from *A. hierochuntica* seeds or from *S. alba* pericarps strongly inhibited or promoted, respectively, bacterial growth (Godwin et al. 2017; Raviv et al. 2017b). We thus sought to examine the effect of substances released from *A. sterilis* husk on growth of the Gram-negative bacteria *Escherichia coli*. *E. coli* was grown in a flat-bottom 96-well microtiter plate in LB medium supplemented with PBS, ampicillin (50 µg/mL) or with substances released from caryopsis 1, caryopsis 2 and the husk of floret 1 and floret 2. Plates were incubated in the dark using a Synergy 4 spectrophotometer (Biotek) and reads (OD_{595}) were taken at 30 min intervals in a course of 12 h. Results showed (Fig. 7) that growth of *E. coli* was accelerated significantly in the presence of husk extracts derived from floret 1 or floret 2 of the *Avena* DUs collected from control C675 plots. No effect on bacterial growth was observed in the presence of extracts derived from caryopsis 1 or caryopsis 2 (Fig. 7a). As expected ampicillin completely inhibited growth of the bacteria. Although, extracts derived from C675 and D180 F1 husks both accelerated bacterial growth, improved bacterial growth was observed with D180 husk extract (Fig. 7b).

Discussion

Due to global warming, it is predicted that water scarcity will increase leading to elevation in drought disaster-affected area in multiple regions of the world (Li et al. 2009). In natural ecosystems, climate warming together with extreme whether events, which are steadily increasing in frequency as well as other land practices such as grazing are predicted to have a serious effect on the structure and composition of plant communities via promoting loss of plant species, shifting species dominance and enabling

Table 1 Differentially present (DP) proteins in the husk of DUs obtained from *A. sterilis* grown in the Mata LTER under control (C675) versus drought (D180) conditions that passed the cutoff of unadjusted *P* value < 0.05 and fold change (FC) > 2

Protein ID	Protein name	Related to At	*P* value	FC (D180/C675)	Category
A0A446NLI4	Chitin-binding type-1 protein	AT3G12500	0.0186	40.3	Response to stress
A0A446Y5T8	Thioredoxin domain-containing protein	AT3G26060	0.000112	30.6	Response to stress
Q41561	Heat-shock protein 16.9C (Fragment)	AT1G53540	0.0233	24.9	Response to stress
P16347	Alpha-amylase/subtilisin inhibitor	AT1G73260	0.0264	21.6	Response to stress
A0A446VD01	Far upstream element-binding protein 2	AT2G25970	0.000227	21.4	Gene expression
A0A446TIH9	Globulin-1 S allele/cupin	AT3G22640	0.0393	18.5	Response to stress
A0A446MKB7	HMA domain-containing protein	AT1G12520	0.000141	17.8	Response to stress
M7YRX7	Putative invertase inhibitor	AT5G45430	0.00808	16.8	Response to stress
A0A446JNB6	Cellulase domain-containing protein	AT5G42100	0.0247	11.8	Carbohydrate process
A0A446YDB8	NTF2 domain-containing protein	AT1G27970	0.0153	11.1	Transport
A0A446NKK9	WD REPEATS containing protein	AT2G19520	0.016	9.58	Response to stress
A0A3B6ELY9	Nucleosome assembly protein 1	AT2G19480	0.00177	7.91	Response to stress
A0A446IIE1	NAD(P)-bd_dom domain-containing protein	AT2G37660	0.00991	6.52	Response to stress
A0A446J2G0	Pyruvate orthophosphate dikinase	AT4G15530	0.0206	5.75	Metabolic process
Q5I7K5	Ribosomal protein P1	AT5G24510	0.047	5.41	Biosynthesis
A0A3B6QJV8	M20_dimer domain-containing protein	AT4G17830	0.0374	5.15	Biosynthesis
T1MVG6	REVERSIBLY GLYCOSYLATED POLYPEPTIDE 2	AT5G15650	0.0246	4.95	Response to chemical
A0A446JFN9	V-type proton ATPase subunit C	AT1G12840	0.0365	4.84	Biosynthesis
A0A446M8N1	PsbP domain-containing protein	AT1G06680	0.0416	4.62	Response to stress
A0A446RR77	LOX5/Lipoxygenase 5	AT3G22400	0.0165	3.69	Response to stress
A0A446L1H5	Aldo_ket_red domain-containing protein	AT1G10810	0.0111	3.69	Redox process
A0A446T632	HATPase_c domain-containing protein	AT5G55200	0.0154	3.49	Response to light
Q7XYD3	Oxygen-evolving complex (Fragment)	AT4G21280	0.0488	3.43	Photosynthesis
A0A446U6W5	Homolog heat-shock protein GrpE	AT4G26780	0.0137	3.28	Response to stress
A0A446NZY4	SBP1 aminotransferase-like	AT4G16050	0.0236	3.27	Biological process
A0A446M3M4	Proteasome subunit alpha type	AT1G16470	0.039	3.25	Response to chemical
A0A446M832	lipid-binding plant phosphoribosyltransferase	AT4G11610	0.0367	2.83	Biological process
A0A3B6ML72	Cysteine synthase	AT3G22460	0.0279	2.74	Response to chemical
A0A077RVD7	Nucleic acid-binding, OB-fold-like protein	AT2G33845	0.0457	2.7	Biological process
A0A446PFC0	Chain A, Triosephosphate isomerase	AT2G21170	0.0413	2.19	Biosynthesis
A0A446JM02	Aminotran_1_2 domain-containing protein	AT4G16050	0.0454	2.15	Biological process
A0A3B6TYR9	Abscisic aldehyde oxidase	AT2G27150	0.0248	− 2.39	ABA biosynthesis process
A0A446VV22	KH domain-BINDING TO TOMV RNA 1	AT5G04430	0.0197	− 2.47	Gene expression
A0A446MSI2	Glucose-6-phosphate 1-epimerase	AT5G57330	0.0272	− 2.69	Response to ABA
A0A446RLE8	NAD_binding_1 domain-containing protein	AT1G20020	0.0167	− 4.68	Response to stress
A0A446MUM9	Chalcone synthase family protein	AT5G13930	0.0316	− 12.3	Response to stress

The related *Arabidopsis* gene ID is given. Fold change between DUs from D180 and C675 plots is presented; (−) indicates downregulation. Category was dissected based on gene ontology (GO) annotations of the *Arabidopsis* genes using GO at TAIR

species invasion (Hellmann et al. 2008; McIntyre et al. 2015; Román-Palacios et al. 2020). Most studies related to plant behavior in response to climate change focused on phenological and physiological processes that eventually affect plant population dynamics and diversity. Yet the most important factor in determining dynamics and diversity of plants is the DU (Walck et al. 2011; Huang et al. 2016; Yi et al. 2019), whose properties particularly the maternal components of the DU, have not been sufficiently studied under variable environmental conditions.

In *Avena* species, the DU constitutes of several florets each composed of husk (lemmas and paleae) that enclosed the caryopsis. The husks are commonly assumed to function in dispersal and in conferring means for embryo protection during storage in the soil and for controlling germination. These maternally derived floral bracts appear to carry out

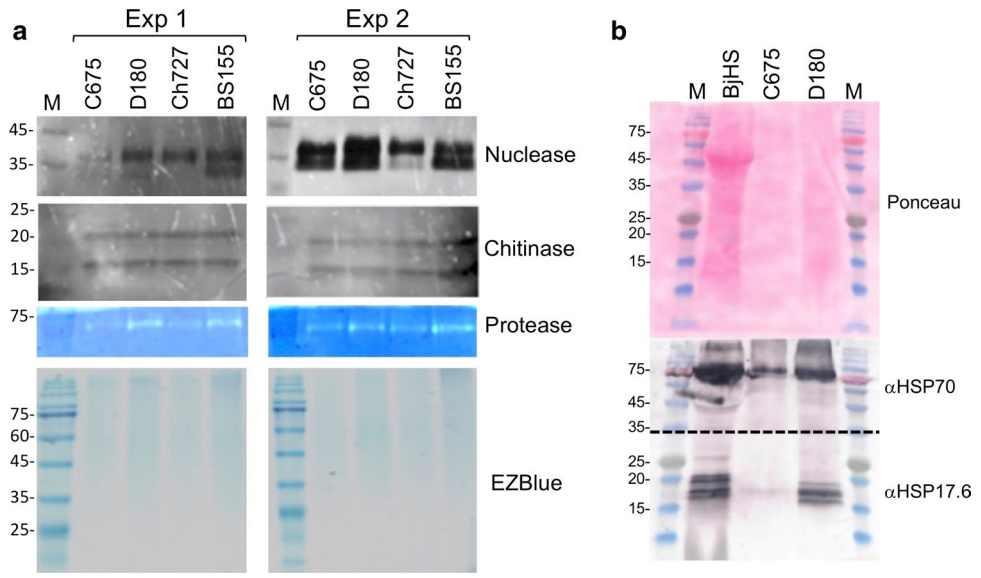


Fig. 5 Analysis of hydrolase activities in *A. sterilis* husk using in gel assays. **a** In-gel assays (Exp1 and Exp2) for nuclease, chitinase and protease activities were performed on proteins extracted from the husk of *Avena* plants in control plots (C675), drought plots (D180) or in natural populations along precipitation gradient from ~ 727 mm (CH727) to ~ 155 mm (BS155). Protein levels loaded in each assay are shown in the EZBlue staining in the lower panel. **b** *Avena* husks from D180 possess high level of small HSP proteins. Proteins from *Avena* husk (C675 and D180) were subjected to immunoblotting using anti HSP70 (αHSP70) and anti-HSP17.6 (αHSP17.6). BjHS is a positive control of proteins extracted from *Brassica juncea* plants subjected to heat stress (3 h 37 °C/7 days). Upper panel is the Ponceau staining of the membrane. Note the membrane was cut into two parts (broken line), the upper containing proteins above 35 kDa was probed with αHSP70 and the lower part with αHSP17.6. M, protein molecular weight markers given in kDa

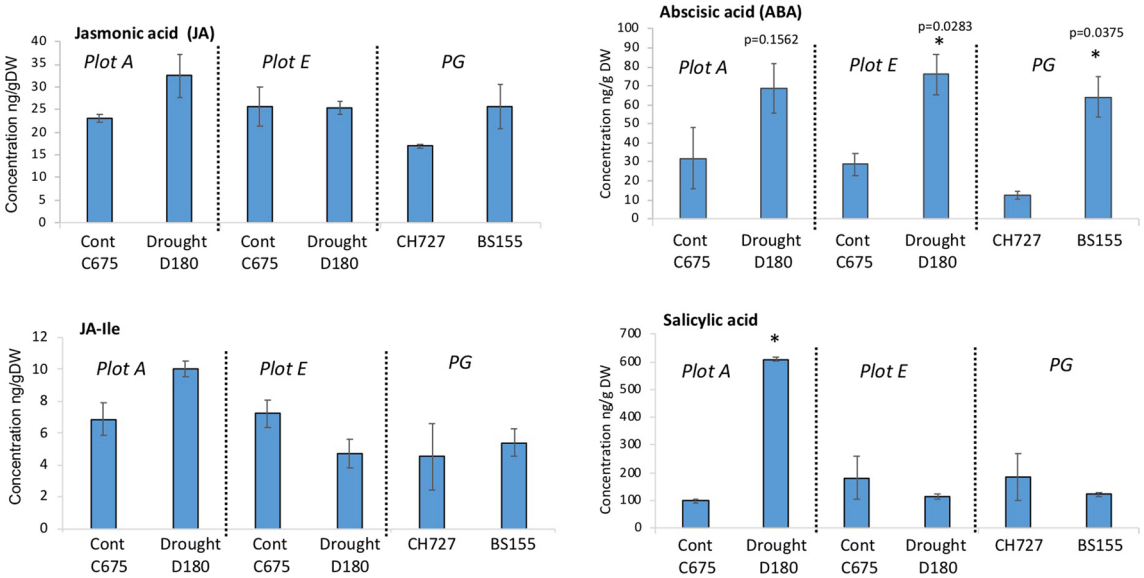


Fig. 6 LCMS analysis of the indicated phytohormones and SA recovered from husk of *A. sterilis* population at the LTER plots (C675 and D180, Plot A and plot E) and other sites along precipitation gradient (PG, Chfetz Haim, CH727 and BS155). Vertical bar is the standard error ($n = 3$). JA-Ile indicates a conjugated JA with the amino acid isoleucine. Asterisks indicate statistically significant differences ($P < 0.05$; Student's unpaired t test)

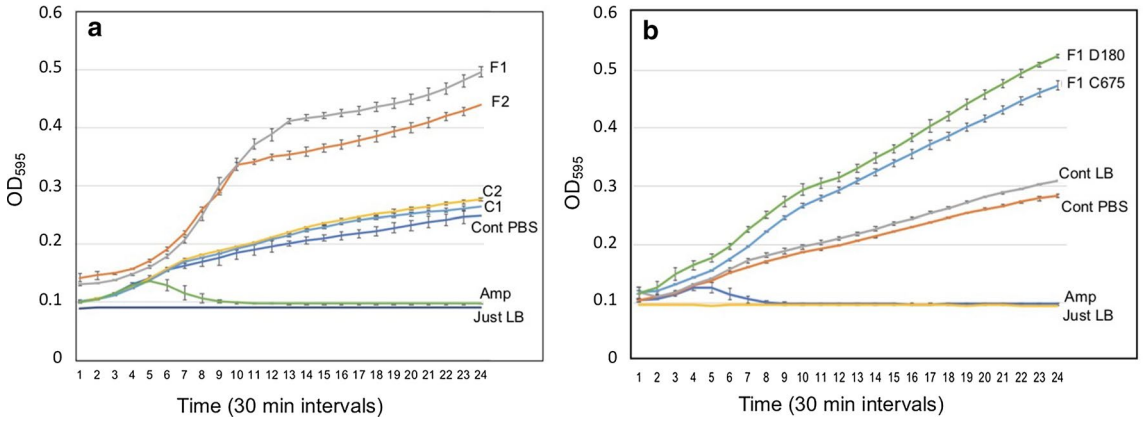


Fig. 7 The husks of *A. sterilis* promote bacterial growth. **a** Analysis of husk and caryopsis extracts of *A. sterilis* from C675 plot. *Escherichia coli* was grown in a flat-bottom 96-well microtiter plate in the presence of PBS (control), or in the presence of substances released from the husk derived from floret 1 (F1), floret 2 (F2), caryopsis of F1 (C1) and caryopsis of F2 (C2). **b** The effect of rainfall on the accumulation of bacterial growth promoting substances in the husks of *A. sterilis* from C675 and D180 plots. *E. coli* was grown in the presence of substances released from the husks derived from floret 1 (F1) of the DU collected at C675 (F1 C675) or at D180 (F1 D180). Ampicillin (Amp, 50 µg/mL) was used as a negative control, PBS and LB are controls and just LB was used as a reference. Bacterial growth was monitored by measuring the OD_{595} of the culture at 30 min intervals in the course of 12 h. Each treatment was performed in triplicates and error bars represent the standard deviation

an elaborated function capable for long-term storage of hundreds of proteins and other substances that can maintain their activities even decades after the death of the organs (reviewed in Raviv et al. 2018). Here we wanted to expand our knowledge on the biological function of the husks, their significance for germination and how the predicted reduction in precipitation, affects the composition and level of substances accumulated within husks in natural populations of *A. sterilis*.

The data presented here clearly show that reduction in water availability had a dramatic effect on the weight of *A. sterilis* DU in natural populations near Beer Sheva (BS155) displaying a 50% decrease compared to Chafetz Haim (CH727) as well as in the LTER site where the DU weight in rainout shelter plots (D180) was decreased by about 30%. Notably, in spite of a significant reduction in caryopsis weights in LTER D180, the final germination was not affected displaying about 80% for both C675 and D180. Also, the germination/emergence index of C675 was similar to that of D180, suggesting that the beneficial effects of the husk on germination capabilities of *A. sterilis* caryopses are preserved regardless of maternal growth conditions. Multiple reports have demonstrated the relationship between seed size and germination capacity. Accordingly, many plants may produce seeds of various sizes often depending on their flowering time, their position on the plant, within the inflorescence, the spike or the spikelet as well as depending on the maternal environment (Fenner 1991; Gutterman 2000; Penfield and MacGregor 2017). Although the final germination level is not affected by seed size, the rate of germination may be affected in a species-specific manner that is, in certain species larger seeds may germinate faster than smaller seeds and vice versa in other plant species (Hendrix 1984; Zhang and Maun 1990). Commonly the rate of seed germination has been addressed with regard to embryo properties and its capacity to execute developmental programs that culminate in the formation of a seedling (Finch-Savage and Bassel 2016). Our data showed that the husks play an important role in determining germination/emergence rate. Germination from the intact DU or the intact floret has a notable advantage over germination from the naked caryopsis irrespective of their DU size. We repeatedly found that germination from the DU is faster, greater and more homogenous than germination from the naked caryopsis both in *A. sterilis* and *T. turgidum* ssp. *dicoccoides*. Fast and homogenous germination might have implications for successful seedling establishment and high vigor in agroecosystems. Similarly, the husks of *Brachypodium hybridum* were shown to have improved germination, germination rate index, as well as seedling growth compared to naked caryopsis (El-Keblawy et al. 2019). Presently, the mechanism(s) by which the husks of wild oat and emmer wheat promote and synchronize germination is not clear. Some of the mechanisms may include mechanical effects where husks may serve as a checkpoint for controlling inflow/outflow of water and gaseous or the protrusion of the radicle or chemical effect due to inflow/outflow of substances (proteins and metabolites) that promote or inhibit germination.

The composition and level of proteins and other substances within the husk of *A. sterilis* may be affected by the growth conditions (precipitation) to which parental plants have been exposed during vegetative growth, flowering and

seed production. Proteome analysis revealed hundreds of proteins released from the husk of *A. sterilis* DU following hydration, with a notable presence of proteins involved in oxidation–reduction process and response to stress. Many enzymes are stored within the husk and might be involved in seed germination and seedling establishment including ROS detoxifying enzymes (Richards et al. 2015) and cell wall-modifying enzymes (Scheler et al. 2015). Many hydrolytic enzymes such as nucleases and chitinases (Supplementary Table S3) may facilitate germination and seedling establishment by defending the growing seedling from potential soil pathogens (Hugot et al. 2002; Sharma et al. 2011). Among the 455 proteins identified, 145 proteins are differentially present displaying at least twofold increase in rainout shelter plots experiencing drought (D180) compared to control plots experiencing 675 mm rainfall (C675); the increase in the level of 31 proteins appears to be statistically significant ($P < 0.05$). Many of these proteins are related to response to stress displaying remarkable increase under growth conditions of reduced water availability including chitin binding type 1 protein (40-fold increase), related to the *Arabidopsis AT3G12500* that encodes a basic chitinase (also known as pathogenesis-related protein 3, PR3) involved in ethylene/JA-mediated signaling pathway during systemic acquired resistance (Chandrashekar et al. 2018). Also, a 30-fold increase in thioredoxin domain-containing protein, a protein related to *Arabidopsis AT3G26060* encoding peroxiredoxin Q, which is induced in response to oxidative stress (Lamkemeyer et al. 2006). The proteome data showed a 25-fold increase in small HSP16.9, related to *Arabidopsis AT1G53540* encoding a member of the class I small heat-shock protein (sHSP) family, which represents the major sHSPs in maturing seeds and might have an importance role in tolerance to multiple types of stresses including heat, salt and oxidative stress (Sun et al. 2002). In addition, we observed a notable increase (~21-fold) in α amylase/subtilisin inhibitor (related to *AT1G73260*), a bifunctional protein that inhibits alpha-amylase, an enzyme responsible for starch degradation, and the activity of subtilisin-type of serine proteases that play a role in defense against herbivores (Arnaiz et al. 2018). It appears that reducing water availability induces the accumulation of multiple stress responsive proteins within the husks of the *A. sterilis* DU, which might provide the seed and the seedling with a primary defense line against both biotic and abiotic stresses.

Metabolic analysis of husks obtained from the LTER control C675 and drought D180 plots as well as from natural population along precipitation gradients revealed the presence of JA and SA that play a major role in plant immunity (Pieterse et al. 2012) and whose levels were not changed significantly in response to changes in rainfall. However, most noticeable is the elevation in ABA in husk from the LTER D180 plots and BS155 experiencing drought. ABA is a well-known stress hormone whose level is commonly increased under various stress conditions (Shinozaki and Yamaguchi-Shinozaki 2007). Both JA and SA might be involved in priming the germinating seeds in preparation to biotic stresses (Worrall et al. 2012; Mauch-Mani et al. 2017). The elevation in ABA, might reflect the physiological state of the plant being responding to water deficit to induce stomatal closure to prevent water loss through transpiration (Wilkinson et al. 2012). Although ABA is well known for its effect on seed dormancy, we could not detect any effect on germination of floret 1 from drought-treated plants (D180 plots) in spite of increased ABA accumulation in the husks. We assume that the increased level of ABA in D180 husks (~75 ng/gDW, which is roughly equal to 3 ng per DU) is not sufficient for inhibition of germination but rather to allow for ABA signaling to induce stress response (Rodríguez-Gacio et al. 2009). Although ABA appears to antagonize the activity of JA and SA in inducing disease resistance, the combined action of these stress regulators at specific concentration levels may be complementary in priming the germinating seeds for both biotic and abiotic stresses (Mauch-Mani and Mauch 2005; Fan et al. 2009).

Interestingly, the husks of *Avena* DUs also release upon hydration allelopathic substances that inhibit, in a species-specific manner, seed germination of *S. alba*, but had no notable effect on seed germination of *B. juncea.* Selective allelopathy described previously (Kushima et al. 1998; Ohno et al. 2001) may have an adaptive value as it can reduce competition for resources by neighboring plant species (Evenari 1949), but at the same time permitting seed germination of species to maintain facilitative plant-plant interaction (Brooker et al. 2008), which might have implications for shaping plant biodiversity and plant community.

We found that the *Avena* husks, but not the caryopses, store and release upon hydration microbial growth promoting substances that strongly enhanced the growth of *E. coli*; caryopsis extracts had no effect on microbial growth. Husks derived from *Avena* DUs from plants experiencing 675 mm and drought (D180) conditions similarly promoted growth of *E. coli.* Depending on the plant species, substances released from DOEEs differentially affect microbial growth. Thus, while strong inhibitory effect on microbial growth was exerted by substances released from *Anastatica hierochuntica* seed coat and pericarps (Raviv et al. 2017b; Khadka et al. 2020), the pericarp of *S. alba* contains substances that promoted microbial growth (Godwin et al. 2017). Inhibitory effect on microbial growth has been reported for grains of sorghum cultivar that inhibit growth and fermentation of *Lactobacillus leichmannii* and *Saccharomyces cerevisiae*; the microbial inhibitor is located in the pericarp fraction (Watson 1975). Similarly, seeds of *Abutilon theophrasti* Medik released a water-diffusible substance(s) that inhibited the growth of many

types of bacteria and fungi (Kremer 1986). The differential effect of DOEEs on microbial growth might be related to the mode of interaction between plants and their unique habitat and the co-evolution with their specific microbiota. We assume that promoting microbial growth by substances released from *Avena* husks might feedback the germinating seeds with bacterial substances such as plant growth regulators and defense inducers (Bérdy 2005) that facilitate survival and plant growth and development.

Conclusions

Together with published data, we highlighted the importance of the DOEEs (e.g., husks) in seed biology and ecology. The DOEEs function as a storage for hundreds of proteins that retain enzymatic activities and other substances including phytohomones, sugars and amino acids as well as allelopathic substances and bacterial growth controlling substances. Obviously, the dead husk represents a functional component of the DU that has been uniquely evolved in each plant species to nurture the embryo and to ensure its success in its unique habitat. Furthermore, we showed that properties of the husks were altered as a result of mother plant exposure to drought. We observed changes in proteins released from the husks and in their enzymatic activities as well as changes in phytohormones levels. Thus, drought conditions that are expected to expand over large areas of the world due to climate change might affect plant diversity and population dynamics not only by modifying embryo properties but also by modifying the properties of the maternally derived DOEEs (e.g., husk), which in turn impact seed performance and fate, soil microbiota and soil fertility (Vandvik et al. 2015; Huang et al. 2016; Doungous et al. 2018).

Author contribution statement BR, JK, SB, JRS, RG, ES and NN conducted experiments and analyzed data. NN and GG, project administration. IG, data analysis, funding acquisition, methodology, reviewing and editing. YG, conceptualization, resources and reviewing and editing. MS, funding acquisition, experimental design, data analysis, reviewing and editing. GG, funding acquisition, wrote the paper, supervision, and data analysis. All authors read and approved the manuscript.

Acknowledgements The work was supported by the NSFC-ISF Research Grant (Grant No. 2456/18 to G.G.) and by the Israeli Ministry of Science and Technology (MOST) (Grant No. 61208 to M.S; ORCID Number: 0000-0001-8710-4141). This work was partly supported by the Joint Usage/Research Center, Institute of Plant Science and Resources, Okayama University, Japan.

References

Adkins SW, Bellairs SM, Loch DS (2002) Seed dormancy mechanisms in warm season grass species. Euphytica 126:13–20

Alon M, Sternberg M (2019) Effects of extreme drought on primary production, species composition and species diversity of a Mediterranean annual plant community. J Veg Sci 30:1045–1061

Arnaiz A, Talavera-Mateo L, Gonzalez-Melendi P, Martinez M, Diaz I, Santamaria ME (2018) *Arabidopsis* Kunitz trypsin inhibitors in defense against spider mites. Front Plant Sci 9:986

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26

Booth DT, Schuman GE (1983) Seedbed ecology of winterfat: fruits versus threshed seeds. J Range Manag 38:387–390

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres L, Kunstler G, Liancourt P, Tielbörger K, Travis JMJ, Anthelme F et al (2008) Facilitation in plant communities: the past, the present and the future. J Ecol 96:18–34

Chandrashekar N, Ali S, Grover A (2018) Exploring expression patterns of PR-1, PR-2, PR-3, and PR-12 like genes in *Arabidopsis thaliana* upon *Alternaria brassicae* inoculation. Biotechnology 8:230

Cousens RD, Young KR, Tadayyon A (2010) The role of the persistent fruit wall in seed water regulation in *Raphanus raphanistrum* (Brassicaceae). Ann Bot 105:101–108

Doungous O, Minyaka E, Longue EAM, Nkengafac NJ (2018) Potentials of cocoa pod husk-based compost on *Phytophthora* pod rot disease suppression, soil fertility, and *Theobroma cacao* L. growth. Environ Sci Pollut Res 25:25327–25335

Elbaum R, Zaltzman L, Burgert I, Fratzl P (2007) The role of wheat awns in the seed dispersal unit. Science 316:884–886

El-Keblawy A, Elgabra M, Mosa KA, Fakhry A, Soliman S (2019) Roles of hardened husks and membranes surrounding *Brachypodium hybridum* grains on germination and seedling growth. Plants 8:322

Evenari M (1949) Germination inhibitors. Bot Rev 15:153–194

Fan J, Hill L, Crooks C, Doerner P, Lamb C (2009) Abscisic acid has a key role in modulating diverse plant-pathogen interactions. Plant Physiol 150:1750–1761

Fandrich L, Mallory-Smith CA (2006) Factors affecting germination of jointed goatgrass (*Aegilops cylindrica*) seed. Weed Sci 54:677–684

Feinbrun-Dothan N, Danin A (1998) Analytical flora of Eretz-Israel. Plitmann U (ed). CANA Publishing House Ltd, Jerusalem

Fenner M (1991) The effects of the parent environment on seed germinability. Seed Sci Res 1:75–84

Finch-Savage WE, Bassel GW (2016) Seed vigour and crop establishment: extending performance beyond adaptation. J Exp Bot 67:567–591

Fukumoto K, Md AK, Yamashita Y, Mori IC, Matsuura H, Galis I (2013) Response of rice to insect elicitors and the role of OsJAR1 in wound and herbivory-induced JA-Ile accumulation. J Integr Plant Biol 55:775–784

Godwin J, Raviv B, Grafi G (2017) Dead pericarps of dry fruits function as long-term storage for active hydrolytic enzymes and other substances that affect germination and microbial growth. Plants (Basel) 6:64

Golodets C, Sternberg M, Kigel J, Boeken B, Henkin Z, Seligman NG, Ungar EU (2015) Climate change scenarios of biomass production along an aridity gradient: vulnerability increases with aridity. Oecologia 177:971–979

Granot G, Morgenstern Y, Khan A, Rapp YG, Pesok A, Nevo E, Grafi G (2015) Internucleosomal DNA fragmentation in wild emmer wheat is catalyzed by S1-type endonucleases translocated to the nucleus upon induction of cell death. Biochim Biophys Acta 1849:239–246

Grover A (2012) Plant chitinases: genetic diversity and physiological roles. Crit Rev Plant Sci 31:57–73

Gutterman Y (2000) Maternal effects on plants during development. In: Fenner M (ed) Seeds: the ecology of regeneration in plant communities, 2nd edn. CABI Publishing, Wallingford, pp 59–84

Hellmann JJ, Byers JE, Bierwagen BG, Dukes JS (2008) Five potential consequences of climate change for invasive species. Conserv Biol 22:534–543

Hendrix SD (1984) Variation in seed weight and its effects on germination in *Pastinaca sativa* L (Umbelliferae). Am J Bot 71:795–802

Hu XW, Wang YR, Wu YP (2009) Effects of the pericarp on imbibition, seed germination, and seedling establishment in seeds of *Hedysarum scoparium* Fisch. et Mey. Ecol Res 24:559–564

Huang Z, Liu S, Bradford KJ, Huxman TE, Venable DL (2016) The contribution of germination functional traits to population dynamics of a desert plant community. Ecology 97:250–261

Hugot K, Ponchet M, Marais A, Ricci P, Galiana E (2002) A tobacco S-like RNase inhibits hyphal elongation of plant pathogens. Mol Plant Microbe Interact 15:243–250

IPCC (2018) Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Masson-Delmotte V, Zhai P, Pörtner H-O, Roberts D, et al. (eds.)

Kader MA (2005) A comparison of seed germination calculation formulae and the associated interpretation of resulting data. J Proc R Soc NSW 138:65–75

Kawaguchi S, Yoneyama K, Yokota T, Takeuchi Y, Ogasawara M, Konnai M (1997) Effects of aqueous extract of rice plants (*Oryza sativa* L.) on seed germination and radicle elongation of *Monochoria vaginalis var. plantaginea*. Plant Growth Regul 23:183–189

Khadka J, Raviv B, Swetha B, Grandhi R, Singiri JR, Novoplansky N, Gutterman Y, Galis I, Huang Z, Grafi G (2020) Maternal environment alters dead pericarp biochemical properties of the desert annual plant *Anastatica hierochuntica* L. PLoS ONE 15:e0237045

Kremer RJ (1986) Antimicrobial activity of velvetleaf (*Abutilon theophrasti*) seeds. Weed Sci 34:617–622

Kushima M, Kakuta H, Kosemura S, Yamamura S, Yamada K, Yokotani-Tomita K, Hasegawa K (1998) An allelopathic substance exuded from germinating watermelon seeds. Plant Growth Regul 25:1–4

Lamkemeyer P, Laxa M, Collin V, Li W, Finkemeier I, Schöttler MA, Holtkamp V, Tognetti VB, Issakidis-Bourguet E, Kandlbinder A et al (2006) Peroxiredoxin Q of *Arabidopsis thaliana* is attached to the thylakoids and functions in context of photosynthesis. Plant J 45:968–981

Li YP, Ye W, Wang M, Yan XD (2009) Climate change and drought: a risk assessment of crop-yield impacts. Clim Res 39:31–46

Lionello P, Abrantes F, Gacic M, Planton S, Trigo R, Ulbrich U (2014) The climate of the Mediterranean region: research progress and climate change impacts. Reg Environ Change 14:1679–1684

Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry–based metabolite profiling in plants. Nat Protoc 1:387

Maere S, Heymans K, Kuiper M (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics 21:3448–3449

Mamut J, Tan DY, Baskin CC, Baskin JM (2014) Role of trichomes and pericarp in the seed biology of the desert annual *Lachnoloma lehmannii* (Brassicaceae). Ecol Res 29:33–44

Mauch-Mani B, Baccelli I, Luna E, Flors V (2017) Defense priming: an adaptive part of induced resistance. Annu Rev Plant Biol 68: 485–512

Mauch-Mani B, Mauch F (2005) The role of abscisic acid in plant-pathogen interactions. Curr Opin Plant Biol 8:409–414

McIntyre PJ, Thorne JH, Dolanc CR, Flint AL, Flint LE, Kelly M, Ackerly DD (2015) Twentieth-century shifts in forest structure in California: denser forests, smaller trees, and increased dominance of oaks. Proc Natl Acad Sci USA 112:1458–1463

Miyajima D (1996) Germination of zinnia seed with and without pericarp. Seed Sci Technol 24:465–473

Nave M, Avni R, Ben-Zvi B, Hale I, Distelfeld A (2016) QTLs for uniform grain dimensions and germination selected during wheat domestication are co-located on chromosome 4B. Theor Appl Genet 129:1303–1315

Ogawa K, Iwabuchi M (2001) A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. Plant Cell Physiol 42:286–291

Ohno S, Tomita-Yokotani K, Kosemura S, Node M, Suzuki T, Amano M, Yasui K, Goto T, Yamamura S, Hasegawa K (2001) A species-selective allelopathic substance from germinating sunflower (*Helianthus annuus* L.) seeds. Phytochemistry 56:577–581

Patton T, Barrett J, Brennan J, Moran N (2006) Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. J Microbiol Methods 64:84–95

Penfield S, MacGregor DR (2017) Effects of environmental variation during seed production on seed dormancy and germination. J Exp Biol 68:819–825

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521

Prakash A, Nithyanand P, Vadivel V (2018) In vitro antibacterial activity of nut by-products against foodborne pathogens and their application in fresh-cut fruit model. J Food Sci Technol 55:4304–4310

Raviv B, Granot G, Chalifa-Caspi V, Grafi G (2017a) The dead, hardened floral bracts of dispersal units of wild wheat function as storage for active hydrolases and in enhancing seedling vigor. PLoS ONE 12:e0177537

Raviv B, Aghajanyan L, Granot G, Makover V, Frenkel O, Gutterman Y, Grafi G (2017b) The dead seed coat functions as a long-term storage for active hydrolytic enzymes. PLoS ONE 12:e0181102

Raviv B, Godwin J, Granot G, Grafi G (2018) The dead can nurture: novel insights into the function of dead organs enclosing embryos. Int J Mol Sci 19:2455

Richards SL, Wilkins KA, Swarbreck SM, Anderson AA, Habib N, Smith AG, McAinsh M, Davies JM (2015) The hydroxyl radical in plants: from seed to seed. J Exp Bot 66:37–46

Rodríguez-Gacio Mdel C, Matilla-Vázquez MA, Matilla AJ (2009) Seed dormancy and ABA signaling: the breakthrough goes on. Plant Signal Behav 4:1035–1049

Román-Palacios C, Wiens JJ (2020) Recent responses to climate change reveal the drivers of species extinction and survival. Proc Natl Acad Sci USA 117:4211–4217

Sankary MN, Barbour MG (1972) Autecology of *Atriplex polycarpa* from California. Ecology 53:1155–1162

Sari A, Oguz B, Bilgic A (2006) Breaking seed dormancy of laurel (*Laurus nobilis* L.). New For 31:403–408

Scheler C, Weitbrecht K, Pearce SP, Hampstead A, Büttner-Mainik A, Lee KJ, Voegele A, Oracz K, Dekkers BJ, Wang X et al (2015) Promotion of testa rupture during garden cress germination involves seed compartment-specific expression and activity of pectin methylesterases. Plant Physiol 167:200–215

Sharma N, Sharma KP, Gaur RK, Gupta VK (2011) Role of chitinase in plant defense. Asian J Biochem 6:29–37

Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. J Exp Bot 58:221–227

Spinoni J, Naumann G, Carrao H, Barbosa P, Vogt J (2014) World drought frequency, duration, and severity for 1951–2010. Int J Climatol 34:2792–2804

Sternberg T (2011) Regional drought has a global impact. Nature 472: 169

Sun W, Van Montagu M, Verbruggen N (2002) Small heat shock proteins and stress tolerance in plants. Biochim Biophys Acta 1577:1–9

Ueno, Miyoshi K (2005) Difference of optimum germination temperature of seeds of intact and dehusked japonica rice during seed development. Euphytica 143:271–275

Vandvik V, Klanderud K, Meineri E, Måren IE, Töpper J (2015) Seed banks are biodiversity reservoirs: species-area relationships above versus below ground. Oikos 125:218–228

Volis S (2014) Dormancy-related seed positional effect in two populations of an annual grass from locations of contrasting aridity. PLoS ONE 9:e93061

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P (2011) Climate change and plant regeneration from seed. Global Change Biol 17:2145–2161

Wang Z, Zhao Y, Zhang Y, Zhao B, Yang Z, Dong L (2019) The role of seed appendage in improving the adaptation of a species in definite seasons: a case study of *Atriplex centralasiatica*. BMC Plant Biol 19:538

Watson TG (1975) Inhibition of microbial fermentations by sorghum grain and malt. J Appl Microbiol 38:133–142

Webb J, Miao S, Zhang X-H (2009) Factors and mechanisms influencing seed germination in a wetland plant sawgrass. Plant Growth Regul 57:243–250

Wilkinson S, Kudoyarova GR, Veselov DS, Arkhipova TN, Davies WJ (2012) Plant hormone interactions: innovative targets for crop breeding and management. J Exp Bot 63:3499–3509

Worrall D, Holroyd GH, Moore JP, Glowacz M, Croft P, Taylor JE, Paul ND, Roberts MR (2012) Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. New Phytol 193:770–778

Wurzburger J, Leshem Y (1969) Physiological action of the germination inhibitor in the husk of *Aegilops kotschyi* Boiss. New Phytol 68:337–341

Xuejun Y, Baskin J, Baskin C, Huang Z (2012) More than just a coating: ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. Perspect Plant Ecol 14:434–442

Yi F, Wang Z, Baskin CC, Baskin JM, Ye R, Sun H, Zhang Y, Ye X, Liu G, Yang X, Huang Z (2019) Seed germination responses to seasonal temperature and drought stress are species-specific but not related to seed size in a desert steppe: Implications for effect of climate change on community structure. Ecol Evol 9:2149–2159

Zhang J, Maun MA (1990) Seed size variation and its effects on seedling growth in *Agropyron psammophilum*. Bot Gaz 151:106–113

Ziv B, Saaroni H, Pargament R, Harpaz T, Alpert P (2014) Trends in rainfall regime over Israel, 1975–2010, and their relationship to large-scale variability. Reg Environ Change 14:1751–1764