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Increased growth in sunflower correlates with reduced defences and altered gene expression in response to biotic and abiotic stress

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Abstract

Cultivated plants have been selected by humans for increased yield in a relatively benign environment, where nutrient and water resources are often supplemented, and biotic enemy loads are kept artificially low. Agricultural weeds have adapted to this same benign environment as crops and often have high growth and reproductive rates, even though they have not been specifically selected for yield. Considering the competing demands for resources in any plant, a key question is whether adaptation to agricultural environments has been accompanied by life history trade-offs, in which resistance to (largely absent) stress has been lost in favour of growth and reproduction. The experiments reported here were designed to test for growth-defence trade-offs in agricultural weeds, crops and native varieties of common sunflower (*Helianthus annuus* L., Asteraceae) by comparing their performance in the presence or absence of abiotic (drought and crowding) or biotic (simulated herbivory, insect herbivory and fungal) stress. We found that growth, as well as viability of crops and weeds, was reduced by abiotic drought stress. The weakened defence in the agricultural genotypes was further evident as increased susceptibility to fungal infection and higher level of insect palatability. To uncover molecular mechanisms underlying these trade-offs, we monitored gene expression kinetics in drought-stressed plants. By correlating phenotypic observations with molecular analyses, we report the identification of several genes, including a protein phosphatase 2C and the HD-Zip transcription factor Athb-8, whose expression is associated with the observed phenotypic variation in common sunflower.

Keywords: agricultural weeds, defence responses, domestication, gene expression, sunflower

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Introduction

The domestication of plants by humans has resulted in the evolution of striking phenotypic changes, including morphologies amenable to high-density growth and harvesting methods and, of course, a physiology that maximizes yield (reviewed in Diamond 2002; Gepts 2004; Hancock 2005). Evolutionary increases in yield necessarily mean that plants are investing more in the particular structures that are desirable to people (e.g. Heiser 1988; Richards 2000; Zohary 2004; Purugganan & Fuller 2009). While agricultural breeding programmes strive to combine high yield with other critical investments – including resistance to stresses such as herbivores, diseases and drought – it is reasonable to assume that some of these investments may trade-off against one another, preventing maximum allocation to all functions (Donald 1968; Halpin 2005). Indeed, life history theory has long suggested that organisms differ largely as a result of how they resolve competing demands on their resources, particularly between growth and other functions (e.g. Pianka 1970; Grime 1977; Tilman 1997). Domestication may represent an

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informative extreme along these trade-offs, where growth investment has been enhanced in the presence of an often artificially benign environment.

If increases in yield have come at the cost of any resistance to stress, the impacts on ecology are likely to be broad. Studies of stress responses in plants have revealed that many defence and tolerance functions are related through shared hormonal responses and metabolic pathways (Singh *et al.* 2002; Fujita *et al.* 2006). For example, Todesco *et al.* (2010) have recently demonstrated that a key regulator in the salicylic acid pathway that enhances plant resistance to microbial infection and herbivory inhibits vegetative plant growth. Thus, it is becoming increasingly clear that stress and metabolic signalling networks interact and that this interaction is important in plant responses to herbivory, pathogen attack, drought, cold, heat and osmotic stresses (reviewed in Cutler *et al.* 2010; Hey *et al.* 2010; Pastori & Foyer 2002; Wilkinson & Davies 2010). This implies that a loss of pathogen resistance could also mean, for example, increased susceptibility to drought. Our understanding of these pathways and their connections is still largely rudimentary, and ecological explorations of the trade-offs among growth and multiple different stress functions are rare. This knowledge gap is a particularly pressing issue for agroecology, as demands on yield are skyrocketing at the same time as pesticide resistance, climate change and reduced water availability impose increasing stresses on crop systems.

Importantly, crops are not the only species that have adapted to the typically benign agricultural environment: so have their weeds. Weeds are well known for their morphological and phenological adaptations for exploiting the cultivated environment and avoiding control measures (Baker 1965; De Wet & Harlan 1975; Stewart *et al.* 2009). In particular, weeds are often larger than their counterparts in natural environments, presumably to compete for light among densely planted crops (Elton 1958; Baker 1965; Crawley 1987). Increased size in weeds might follow the same trade-offs as high yield in domesticated plants, with the same associated costs to stress resistance. In fact, the loss of certain types of stress resistance (especially defences against natural enemies) is one of the dominant hypotheses for how increased growth and/or reproduction is achieved in weedy species (Keane & Crawley 2002; Daehler 2003; Colautti *et al.* 2004).

The common sunflower (*Helianthus annuus* L., Asteraceae) provides an outstanding opportunity to evaluate such trade-offs between stress resistance and growth, because different forms of the same species exist as a domesticated crop, an agricultural weed and a wild species in natural areas. Sunflower is native to open grasslands in central and western North America (Heiser *et al.* 1969) and was domesticated over 4000 years ago for seed production (Harter *et al.* 2004). While wild sunflower produces short, branched stems and many small flowering heads, domesticated sunflowers produce a tall unbranched stem and a single very large head. A third and weedy form of sunflower has become a pest in croplands and highly disturbed areas and is found throughout North America (Massinga *et al.* 2003; Kane & Rieseberg 2007), Argentina (Poverene & Cantamutto 2010) and in much of Europe (Faure *et al.* 2002; Holec *et al.* 2005; Vischi *et al.* 2006; Muller *et al.* 2009). Sunflower weeds in agricultural fields have a morphology generally intermediate between wild and domesticated varieties with tall but branched stems (Muller *et al.* 2009). This weedy form appears to have arisen multiple times from nearby native populations in North America (Kane & Rieseberg 2007) and to have been influenced by hybridization with cultivated plants in Europe (Muller *et al.* 2010).

There are strong indications that stress- and defence-related responses might play a central role in the genetic differences among these varieties. A genomic scan for evidence of selection in weedy populations identified a heat shock gene with independent sweeps across several weedy sunflower populations (Kane & Rieseberg 2007). A comparative analysis of gene expression in weedy and wild populations also revealed that most of the differentially expressed loci are defence- and stress-related (Lai *et al.* 2008). In the current study, we specifically examine the relationship between growth and phenotypic responses to various stresses in 21 native, weedy and domesticated populations. We also explore gene expression responses to drought stress in these populations. Our findings largely support the trade-off hypothesis as a mechanistic basis for the growth differences among the varieties of sunflower and suggest a role of specific candidate genes in the observed trade-offs.

Materials and methods

Plant material

Seeds of seven native sunflower populations were collected from uncultivated grasslands at Trout Creek, Utah; Eureka Springs, Arkansas; Moose Jaw, Saskatchewan; Denver, Colorado; Colby, Kansas; De Soto National Wildlife Refuge, Iowa; and Santa Rosa, New Mexico. Seeds of seven weedy populations were collected from within intensively cultivated corn, wheat and pumpkin fields at Davis, California; Indianapolis, Indiana; Norton, Kansas; Delta, Utah; Veszprém, Hungary; Vertiskos, Greece; and Cordoba, Spain. Collection details are available from the authors upon request.

Populations from which seeds were collected comprised at least 500–10 000 individuals. Domesticated lines were obtained from USDA germplasm repository in Ames, Iowa, and included five Native American cultivars (Hidatsa, Hopi dye, Mandan, Nagyatadi and Seneca) and two modern cultivars (Comet and HA89).

Treatments and assays

Seeds of the 21 populations described earlier were germinated in the laboratory, and seedlings were grown in the greenhouse at 22–25 °C with a 16-h light cycle. For each analysis, 4–6 individuals from bulk collections with mixed maternal families for each population were used. Experiments were conducted ~4 weeks after germination, when seedlings had 4–8 true leaves. Control plants were grown under standard greenhouse conditions throughout the experiments. All experiments reported here represent at least two independent replications.

Drought

Extreme water deficit was induced by withholding water. Growth, measured as plant height, total number of leaves and length and width of the longest leaf, was estimated before treatment and after 7 days of stress. Statistical analysis was performed in JMP (Version 7; SAS Institute Inc., Cary, NC, USA, 1989–2007). Biological replicates were included as temporal blocks and were treated as a covariate. Analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to assess the effects of group (the fixed effect) and population (random effect nested within group) on plant growth in height. Interactions between plant group and treatment (including the control, drought, crowding and simulated herbivory treatments) were also tested.

Survival, measured as number of days to first appearance of wilting symptoms and days to plant death, was recorded every day throughout the experiments. Univariate survival analyses, testing for group effects on days to wilting and days to plant death, were conducted in JMP using the product-limit method (Kaplan & Meier 1958) with *P*-values determined by the Wilcoxon test. Associations between different phenotypic measures (including plant height, number of leaves before treatment, number of new leaves during treatment and days to plant death) were tested using linear regressions.

Crowding

To create crowding stress, plants were spaced <5 cm apart, which is at the upper end of densities found in natural populations (Cummings & Alexander 2002). Growth, measured as plant height, total number of leaves and length and width of the longest leaf, was estimated before treatment and once a week for three consecutive weeks. Statistical analysis was conducted using ANOVA as detailed for the drought treatment.

Simulated herbivory

To simulate herbivory stress, plants were sprayed with 1 mM methyl-jasmonic acid (Sigma-Aldrich) as described previously (Martin *et al.* 2003). Spraying was carried out to obtain a complete and even coating. Additionally, the longest leaf of each plant was cut in half. This treatment was performed once a week for three consecutive weeks. Growth, measured as plant height, total number of leaves and length and width of the longest leaf, was estimated before treatment and after 2 weeks of stress. Statistical analysis was conducted using ANOVA as detailed for the drought treatment.

Herbivory

Plants were tested for feeding deterrence effects by paired choice bioassays (Akhtar *et al.* 2003) using the generalist herbivore *Trichoplusia ni*, which feeds on a wide range of flowering plants including sunflower. *Trichoplusia ni* is distributed throughout the world, covering the range of all three groups of sunflowers. We used an established (>50 generations) laboratory colony reared at room temperature (19–25 °C) under a 16:8 L:D photoperiod. Larvae were reared on an artificial diet (No. 9795; BioServ Inc., Frenchtown, NJ, USA), supplemented with finely ground alfalfa to improve acceptability. Adult *T. ni* were supplied with a 10% sucrose solution accessed by a cotton wick in a sealed 100-ml plastic container. Fresh leaf discs were pooled from each plant population (typically 4–6 plants), and larvae of *T. ni* were given the choice of feeding on two leaf discs: one native and the other one weedy or domesticated. Leaf areas consumed by the insects were measured, and feeding deterrence index (FDI) was calculated as described in Akhtar *et al.* (2003); positive values indicate insect preference for the native leaf dick, while negative values indicate preference for the weedy or domesticated dick. Mean FDI values were calculated for each pairwise comparison, yielding a distribution of FDI values for the overall comparison of native to either weedy or domesticated plants. One-sample *t*-tests were then used to assess the deviation of these distributions from the null hypothesis of zero, i.e. no preference (normality was verified using quantile–quantile plots).

Fungal infection

Susceptibility to fungal infection was assayed using applications of the generalist fungal pathogen *Botrytis cinerea*, which is known to infect sunflowers (Williamson *et al.* 2007) and damage plants to the extent that it limits the cultivation of sunflowers in certain regions (Crowley 1998; Moschner & Biskupek-Korell 2006). *Botrytis cinerea* was cultured on 2 × V8 agar (36% V8 juice, 0.2% CaCO3, 2% Bacto-agar) under ambient laboratory conditions (cool-white fluorescent lamps, 12-h photoperiod and temperature range of 21–24 °C). For inoculations, conidia were collected from 2-week-old cultures and resuspended in 0.01% Tween 20 to a final concentration of 2×10^6 conidia per ml. Native, weedy and domesticated sunflowers were inoculated by placing a 1 mL droplet of conidia suspension on each leaf. Spread of infection was monitored for 6 days and scored as primary infection (i.e. symptoms restricted to the cells that came in contact with the spore suspension), secondary infection (i.e. some spread to adjacent cells and through to the other side of the leaf but not throughout the leaf) or tertiary infection (i.e. the majority of the leaf was infected). Analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to assess the effects of group (the fixed effect) and population (random effect nested within group) on infection levels at each day of the experiment.

Gene expression analysis

A preliminary gene expression analysis was performed with 23 stress-related genes to monitor mRNA differences between untreated (control group) and drought-stressed (experimental group) tissue at a single time point during drought response (day 6 post-treatment). These candidate genes were chosen based on multiple sources previously investigating gene expression changes in sunflowers upon stress challenges (references detailed in Table S1, Supporting information). In addition, two stress-related genes that were found to be downregulated in weedy populations under benign conditions (Lai *et al.* 2008) were included in our preliminary analysis.

Forty-five individuals were grown from each of the following plant populations: Utah native, Kansas native, Colorado native, California weedy, Utah weedy, Greece weedy, Hopi dye cultivar, Mandan cultivar and HA89 cultivar. Young, fully expanded leaves were sampled, immediately frozen in liquid nitrogen and stored at −80 °C for 1–3 weeks. Total RNA was extracted from these frozen samples using the TRIzol reagent (Invitrogen)/RNeasy (QIAGEN) approach as described previously (Lai *et al.* 2006). RNA in the extracted samples was quantified using spectrophotometry (NanoDrop). Total RNA was treated with RNase-free DNase I (QIAGEN) to eliminate genomic DNA contamination, and sample quality was tested on an agarose gel as well as by a standard PCR (without reverse transcription).

First-strand cDNA was synthesized with Superscript III (Invitrogen). qRT–PCRs were performed in iQ5 real-time PCR detection system (Bio-Rad). The quantitative RT–PCR analysis was biologically repeated three times, and each time consisted of three technical replicates. The reaction contained template cDNA, 200 nM gene-specific primers (as specified in the corresponding references, Table S1, Supporting information) and iQ SYBR Green supermix (Bio-Rad) in a total volume of 15 μL. Reactions were carried out with the following cycling programme: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 54 °C and 30 s at 72 °C. Data were collected at 72 °C in each cycle, and the absence of nonspecific products and primer dimers was confirmed by analysis of melting curves and agarose gel electrophoresis. We used the normalized expression method (ddCt) by assigning two independent reference genes as internal standards: actin (accession no. AB199316) and 60S ribosomal protein (accession no. QH_CA_Contig1047_1). Amplification efficiency was determined from the slope of the log-linear portion of the standard curve and was above 95% for both the genes of interest and the reference genes. In addition to the test samples, each qRT–PCR run included a no-template control sample for assessing reliability of amplification, as well as a 5-log serial dilution of a known sample (in triplicates) for generating a standard curve as an indication of the analytical sensitivity and the robustness of the assay. Seven genes, for which this preliminary analysis suggested differential expression between the three plant groups prior to and/or during the drought response (labelled with * in Table S1, Supporting information), were further used for a detailed study of expression kinetics. For this aim, plants were exposed to progressive drought treatment as described earlier, and leaf tissue was collected before treatment and on days 2, 4, 6 and 8 post-treatment for total RNA isolation.

Analysis of gene expression data was conducted in R (http://www.R-project.org) at individual time points after the cessation of watering and for the entire reaction norm of expression changes with time. We searched for expression differences that occur at a specific time point by comparing the three plant groups (natives, weeds and crops) at each time separately. Mixed effects models (R package lme4, function lmer, Bates & Maechler 2010) were used with plant group as the fixed effect and with random effects corresponding to the different populations nested within groups and

to the replicates. For some time points, the data are unbalanced. The *P*-values were calculated using a *t*-distribution with *k* degrees of freedom, where *k* = (number of different groups − number of fixed effects).

In the reaction norm analysis, we integrated all five time points to compare the overall expression pattern during the time-course of the experiment. We used a linear mixed effects model (R package lme4, function lmer, Bates & Maechler 2010) with random slopes and intercepts for the different populations nested within groups. The effect of group is a change in intercept and is treated as a fixed effect. The replicate effect was included in the analysis as a random effect. The *P*-values were calculated by parametric bootstrap.

Results

Plant growth and survival

Phenotypic differences among weedy, native and domesticated sunflowers were apparent in our different growth conditions. Under the control treatment (intact plants), there was a significant difference in growth of the three plant groups (*P*-value <0.0001), mainly driven by the domesticated lines growing the most quickly (*P*-value <0.01 for the comparison of domesticated plants to either natives or weeds). Growth of the native plants was the slowest, although statistically indistinguishable from that of weeds (Fig. 1).

We next tested for growth differences under three stress conditions, including drought, crowding and simulated herbivory. Of the various stresses, drought had the largest effect on plant growth (*P*-value <0.0001 for the comparison of drought with control; other treatments NS). Upon drought, growth of the domesticated and weedy plants was significantly reduced as compared to the control (*P*-value <0.001 for domesticated and *P*-value <0.01 for weeds), while that of native plants was only marginally affected (*P*-value = 0.08). The significant interaction effect of treatment by group (*P*-value <0.001) resulted in comparable growth of the three plant groups under this stress treatment (*P*-value >0.1; Fig. 1).

To further examine differences in the response of the three plant groups to prolonged drought, we recorded plant survival, determined by the number of days to plant death (days to the first appearance of wilting symptoms yielded similar results). As evident in

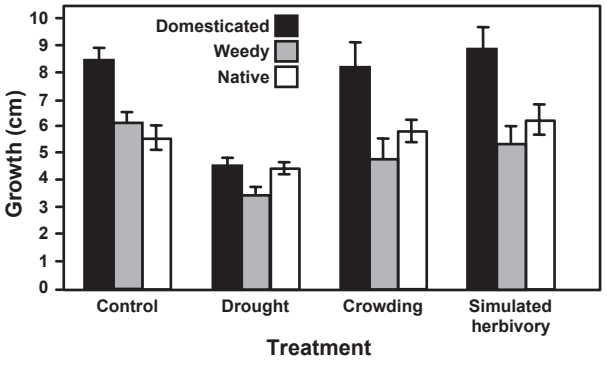

Fig. 1 Growth of native, weedy and domesticated populations. Plants of native, weedy and domesticated populations were exposed to drought, crowding or simulated herbivory stress. Growth rates (±SE) are presented as total change in plant height. Each point is an average of seven different populations.

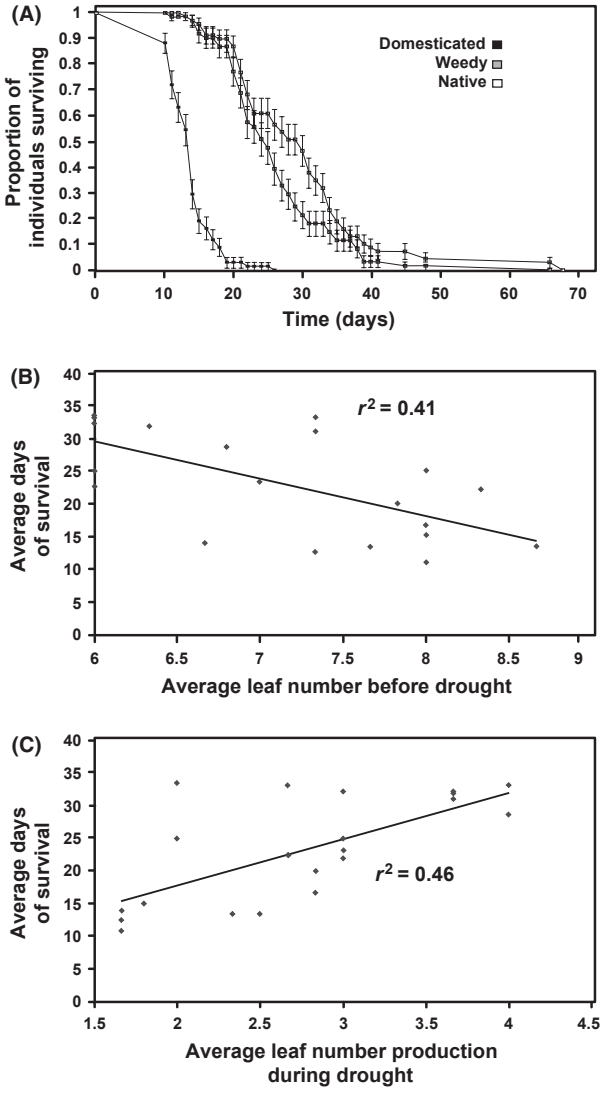

Fig. 2 Survival rates of native, weedy and domesticated populations under progressive drought stress. (A) Proportion of surviving individuals in each plant group (±SE) was documented throughout the experiments. Correlations are shown between (B) survival times and pretreatment leaf number and (C) number of new leaves produced during the experiment.

Fig. 2A, the three plant groups exhibited significantly different survival times (P-value <0.0001). The domesticated plants showed the shortest survival, with 97% of the plants already dead by day 20 of the drought treatment. At this time point, most (>88%) native and weedy plants did not yet show any wilting symptoms. The difference between native and weedy plants became apparent at a later time point in the drought response, with natives exhibiting significantly longer survival times than weeds (P-value <0.05; Fig. 2A). In general, plants that grew in height more during the experiment died more rapidly (r^2 = 0.29, P-value <0.05; Fig. S1, Supporting information). Nevertheless, native plants survived for much longer time periods despite having comparable growth rates to domesticated lines under drought conditions (Fig. 1). Interestingly, while plants with fewer pretreatment leaves survived longer (r^2 = 0.41, P-value <0.01; Fig. 2B), growth of new leaves during the drought response was associated with longer survival (r^2 = 0.46, P-value <0.001; Fig. 2C).

Herbivory defence

Leaf choice bioassays (Akhtar *et al.* 2003) were conducted to determine antifeedant effects of native, weedy and domesticated sunflower populations. Figure 3A presents the obtained values in a two-colour matrix, where red boxes indicate relative preference for the native leaf disk (strongest preference is light red), green boxes indicate relative preference for the weedy or domesticated disk (strongest preference is light green), and black boxes indicate no preference. Evidently, the *Trichoplusia ni* insects showed a general preference for leaves from weedy and domesticated plants over native ones (P-value <0.05 for weeds and P-value <0.01 for domesticated; t-test). These results suggest a lower level of feeding deterrence effects in both weedy and domesticated plants as compared to native ones. Notably, several native populations (such as Colorado, Saskatchewan and Arkansas) were left unconsumed in almost every comparison. Weedy lines from Europe (i.e. Hungary, Greece and Cordoba) were almost exclusively preferred by the larvae in all paired choice bioassays and weedy lines from California and Indiana were mildly preferred, while weedy populations from Kansas and Utah were consumed less by the insects.

Fungal stress

Plant inoculations with *Botrytis cinerea* lead to marked differences in the progress of infection between native, weedy and domesticated sunflowers. On day 5, when secondary infections were evident, there were significant differences between domesticated and native

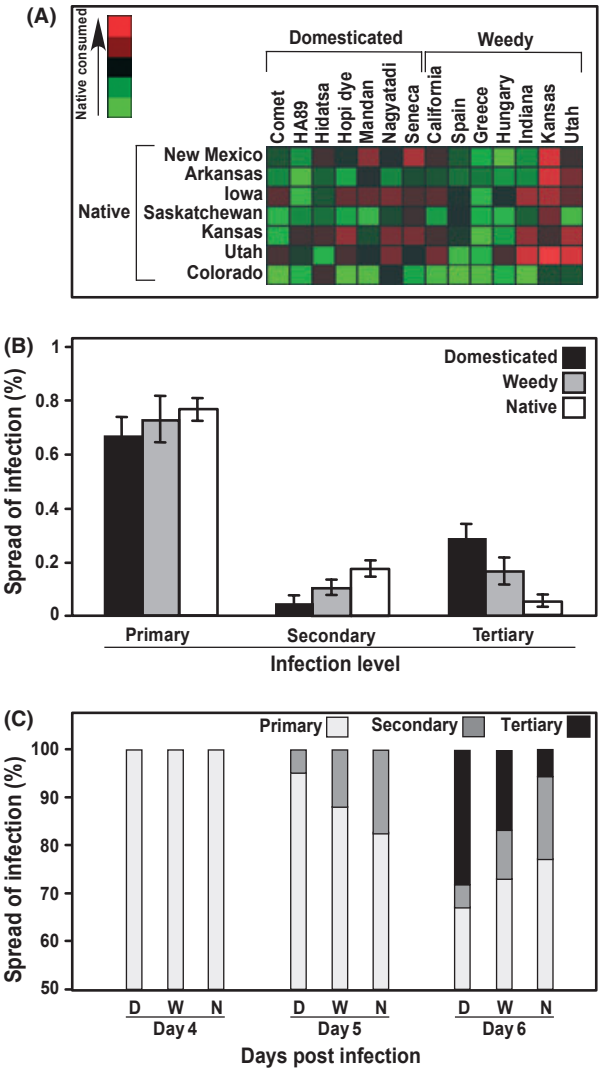

Fig. 3 Response of native, weedy and domesticated populations to biotic stress. (a) Feeding deterrence effects on the *Trichoplusia ni* herbivore: Larvae were given the choice of feeding on two leaf discs, one native (as detailed on left side of coloured panel) and the other one domesticated or weedy (detailed above panel). The colour scale at the upper left corner shows feeding preferences, where red indicates insect's preference for native leaf discs, green indicates insect's preference for weedy/domesticated leaf discs, and black indicates no preference. (b) Grey mould disease spread (±SE) on day 6 postinfection with *Botrytis cinerea*: Plants were inoculated with *B. cinerea* and spread of infection was scored as primary, secondary or tertiary (as detailed in 'Materials and methods'). (c) Spread of *B. cinerea* infection on days 4, 5 and 6 postinoculation.

plants, with the latter showing more secondary infections (P-value <0.05; Fig. 3C). Weedy plants were intermediate, not significantly different from either native or domesticated plants. On day 6, many plants exhibited tertiary infections, with infection progressing differently

in the three groups. As evident in Fig. 3B, at this point, domesticated plants showed lower rates of secondary infections (P-value <0.01) but significantly higher rates of tertiary infection (P-value <0.01) compared with native plants, with the weedy plants again intermediate and not significantly different from either group.

Transcriptional changes in response to drought

The variation in growth and survival upon drought stress between native, weedy and domesticated plants prompted us to investigate these differences at the molecular level.

Of seven candidate genes, for which our preliminary expression analysis suggested transcriptional regulation during drought response (see 'Materials and methods'), four genes exhibited significant correlation between mRNA level and the number of days to plant death ($r^2 > 0.35$, P-value <0.01; Fig. 4). These four genes were then investigated by a reaction norm analysis to examine whether gene expression dynamics differ between the three plant groups over time. This analysis revealed a plant group effect in the expression of two of these genes: a protein phosphatase 2C and the HD-Zip transcription factor Athb-8.

For the protein phosphatase 2C gene, the effect was mainly driven by the different expression pattern found in domesticated vs. native plants (P-value <0.05; Fig. S2, Supporting information). This gene belongs to a family of serine/threonine protein phosphatases that play an important role as negative regulators in plant responses to environmental stresses such as drought (reviewed in Schroeder *et al*. 2001; Tahtiharju & Palva 2001). For the gene encoding the HD-Zip transcription factor Athb-8, there was significantly different kinetics between domesticated and either natives (P-value <0.05) or weeds (P-value <0.01; Fig. 5). HD-ZIP proteins have been proposed to trigger developmental responses to environmental conditions in plants (Schena & Davis 1992). In our experiments, expression of the Athb-8 gene was downregulated during the drought response in all three plant groups, with the least repression observed in native plants (fold repression 1.3), followed by weeds (2.6) and the highest repression found in crops (3.2). These results may suggest a role of the Athb-8 transcription factor in the reduced growth and survival of domesticated and weedy plants during drought. The finding that mRNA kinetics of the Athb-8 gene differs among the three plant groups prompted us to further examine the expression of this gene for each time point separately. Interestingly, no plant group effect was found for the expression of the Athb-8 gene in control plants, i.e. prior to the drought treatment. Yet significant differences between the three plant groups developed during the drought response and throughout the experiment; the mRNA level of the gene in domesticated lines was significantly different from that in native plants at all time points analysed (P-value <0.05) and significantly different from that of weeds on days 2 and 4 post-treatment (P-value <0.05).

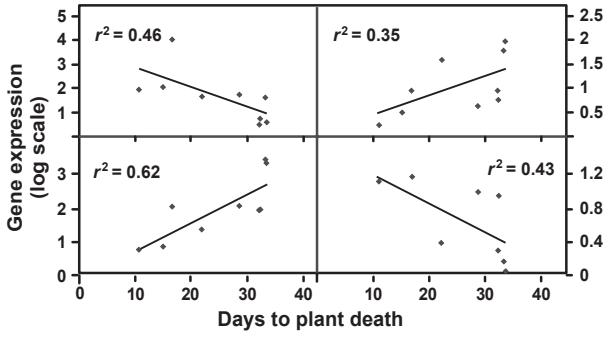

Fig. 4 Expression analysis of stress-related genes during drought response. Plants were exposed to water deficit, and harvested frozen tissue was used for RNA extraction and subsequent cDNA synthesis. Transcript levels of the genes encoding osmotic stress–activated protein kinase (GenBank ID CX945586, upper left panel), HD-Zip transcription factor Athb-8 (GenBank ID AJ412547, lower left panel), protein phosphatase 2C (GenBank ID CD847464, upper right panel) and HaF455 (GenBank ID BU672004, lower right panel) were monitored by qRT–PCR. Relative expression is expressed as delta-delta-Ct normalized to 60S rRNA and actin expression. Correlations are shown between mRNA level and the number of days to plant death.

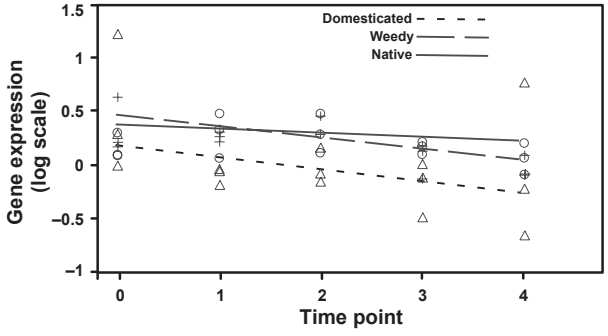

Fig. 5 Expression analysis of the Athb-8 gene during drought response. Transcript level of the gene encoding the HD-Zip transcription factor Athb-8 (GenBank ID AJ412547) was monitored by qRT–PCR (three technical replicates per biological replicate) several times during drought response in weedy (plus symbols), domesticated (triangles) and native (circles) plants. Relative expression is expressed as delta-delta-Ct normalized to 60S rRNA and actin expression.

Discussion

Trade-offs in resource allocation are expected to fundamentally shape the evolution of physiological, morphological and life history traits (Stearns 1976; Van Noordwijk & Dejong 1986). Among domesticated plants, selection for growth traits that generate higher yields has almost certainly come at a cost to other functions (Donald 1968; Massei & Hartley 2000). Here, we suggest that trade-offs between growth and defence response functions are likely, both because they can be costly (Mole 1994; Stowe *et al.* 2000) and because the crop environment is intentionally controlled to be as benign as possible for plant growth. Similarly, we expect that the benign crop environment will also allow agricultural weeds to have increased growth at the expense of defence response traits.

We tested for such negative correlations between growth and defence response across weedy, domesticated and native sunflower genotypes. Indeed, we found that growth traits measured under benign (control) conditions tended to be greater for the domesticated genotypes than for the native genotypes. Moreover, we were able to detect reduced defence abilities in both the weedy and particularly domesticated sunflowers upon exposure to biotic and abiotic stressors, consistent with the trade-off hypothesis.

Among the biotic stresses that we examined, both herbivore choice tests and fungal infection assays revealed weakened defences in weeds and crops, especially the latter. Our experiments with the generalist herbivore *Trichoplusia ni* clearly demonstrated higher palatability of both weedy and domesticated populations compared with native plants. It is possible that the higher growth rate and palatability of some plants are simply associated with higher nutritional value for the *T. ni* herbivore (Mattson 1980; Cornelissen *et al.* 1997). Alternatively, because chemicals that deter feeding of phytophagous insects are an integral and resource-costly part of the plant defence system (Stowe *et al.* 2000; Eric *et al.* 2003; Von Dahl *et al.* 2007), larval preference for these genotypes may reflect a weakened defence system. High levels of defensive secondary compounds found in sunflower leaves can slow the growth and development of lepidopteran herbivores (Rossiter *et al.* 1986). Unsurprisingly, these herbivores prefer leaves with lower levels of such compounds. Domesticated sunflowers are also preferred over wild sunflowers by lepidopteran pests in experimental agriculture fields (Chen & Welter 2005). Other studies have shown that native sunflower populations are under selection for resistance to local lepidopteran herbivore communities, reflecting the importance of maintaining these traits in nonagricultural settings (Whitney *et al.* 2006). Inoculation of plants with the fungal pathogen *Botrytis cinerea* also revealed more severe effects on weedy and domesticated sunflowers. While native plants more readily developed secondary infection, tertiary infection was limited mainly to weedy and particularly to domesticated plants. Importantly, plants can deploy several inducible defences that slow disease spread by limiting fungal growth around the infection site. One such mechanism is the induction of necrosis (and the accompanying defence response), which effectively restricts fungal expansion (Elad & Evensen 1995). This suggests that the rapid secondary infection observed in native sunflowers was associated with an efficient defence mechanism, preventing the tertiary infection rates seen in the (more susceptible) weedy and domesticated genotypes.

The agricultural genotypes also proved to be more sensitive to an abiotic stress: drought. Specifically, growth of weedy and particularly domesticated plants was significantly reduced upon drought conditions and they wilted earlier than their native counterparts. Not surprisingly, larger plants, which have relatively more water-consuming tissue and larger leaf surface area from which to lose water, also died earlier upon drought. Previous studies have similarly found that plants with relatively low growth rates survive for longer time periods during drought (Givnish 1979; Zangerl & Bazzaz 1984; Donovan & Ehleringer 1992; Ehleringer 1993; Dudley 1996; Heschel *et al.* 2002). In our case, however, even after controlling for the effect of plant size, significant differences remained between survival of native, weedy and domesticated plants. Moreover, emergence of new leaves during the stress response was associated with higher plant viability. It is well known that to reduce the adverse effects of drought stress, plants have evolved multifaceted strategies, including morphological, physiological and biochemical adaptations (Ingram & Bartels 1996; Xiong & Zhu 2002; Bohnert *et al.* 2006). It is possible that abscising existing leaves and forming new leaves with different morphology and/or drought-adapted characteristics allow (and reflect) higher drought response abilities in native sunflowers.

In contrast to drought, domesticated plants showed little response to both crowding and simulated herbivory. It is possible that drought was the most extreme treatment (i.e. it was the only treatment leading to plant death), while the other treatments were not severe enough. Alternatively, perhaps cultivated sunflowers have been (inadvertently or intentionally) selected to tolerate crowding and jasmonate-induced stresses, or their tolerance is a by-product of selection on domestication-related traits (De Wet & Harlan 1975; Sawers *et al.* 2005). Our findings that different sunflower

varieties were not affected by exogenous application of jasmonic acid may suggest reduced sensitivity of sunflowers to jasmonates with respect to growth, an interesting finding that merits further research. Increased concentrations and/or different timing of application of the chemical should also be explored for their effect on growth.

Our discovery of variation in drought-related defence abilities of native, weedy and domesticated sunflowers prompted us to investigate these differences at the molecular level. Gene expression is often a major contributor to biological novelty and appears to underlie most phenotypic variation (e.g. Doebley *et al.* 1995; Enard *et al.* 2002; Osborn *et al.* 2003; Wray 2003; Carroll 2005; Clark *et al.* 2006; Gilad *et al.* 2006; Haerty & Singh 2006; Khaitovich *et al.* 2006; Prud'homme *et al.* 2006; McGregor *et al.* 2007; Lai *et al.* 2008). By performing a comparative study of gene expression kinetics among populations, we identified changes in the expression of four genes that correlate with the observed phenotypes, suggesting that they may coordinately play roles in the underlying cellular defence pathway/s. In agreement with previous studies (John 2001; Srikanthbabu *et al.* 2002), variability in gene expression was only evident upon exposure to the applied stress. Three of these genes – osmotic stress–activated protein kinase, HD-Zip transcription factor Athb-8 and protein phosphatase 2C – were previously associated with drought response (Roche *et al.* 2007, 2009), while the fourth –HaF455 – was reported to be induced upon cold and salinity stresses (Fernandez *et al.* 2008) but was not previously associated with drought response.

The highest correlation between expression level and drought phenotype was evident at different time points for the four different genes. It is now well established that an efficient defence response to drought depends on complex regulatory signalling networks and the orchestrated activity of genes with diverse cellular functions (e.g. Asselbergh *et al.* 2008; Cutler *et al.* 2010). As specific functions are required at specific points during a given response, the timing of genes' expression is strictly regulated. The candidate genes identified in the current study belong to different functional categories, including transcription (HD-Zip transcription factor Athb-8), signal transduction (osmotic stress–activated protein kinase and protein phosphatase 2C) and ribosomal activity (HaF455). It is thus not surprising that these loci show unique patterns of activity during the stress response.

Of the four genes that exhibited correlation between gene expression and drought phenotype, a gene encoding the Athb-8 transcription factor was found to be kinetically different between the plant groups at all time points. Expression of this gene appeared to be consistently correlated with the observed differences in response to drought, and additional work to examine the regulatory source of these differences and their prevalence across other populations (and other species) would help to establish its importance to the evolution of weeds and domesticates. For example, expression analyses of F1 hybrids of domesticated and weedy/native genotypes could be used to elucidate whether the functioning of Athb-8 in drought response is *cis*- or *trans*-regulated (Wittkopp *et al.* 2004; Springer & Stupar 2007), and microarray surveys (ongoing) of drought-stressed sunflower genotypes will also aid in determining the regulatory basis of this gene's function.

Two of the genes whose expression was associated with the differential drought phenotypes were previously shown to respond to a range of stresses and environmental cues (Athb-8, Baima *et al.* 1995; HaF455, Fernandez *et al.* 2008). Convergence between different defence pathways is very common in plants; under natural conditions, many stresses occur at once, and plants have evolved defences against biotic and abiotic stresses that may co-occur in complex environments (Pastori & Foyer 2002; Cutler *et al.* 2010; Wilkinson & Davies 2010). In support of this idea, in the current study, we observed reduced defences to both biotic and abiotic stresses in weeds and crops.

Together, our analyses make a clear case for reduced defences in domesticated and, to a lesser extent, weedy sunflowers, demonstrating the potential for broad consequences of increased resource allocation to individual traits like growth and supporting the hypothesis of fitness trade-offs in these plants. To confirm the action of these trade-offs with certainty, the next challenge is to identify the genetic basis of variation in both growth and stress responses and to determine how resource allocation is shifted among these functions. Association mapping and surveys of growth and stress traits in recombinant inbred lines, derived from crosses of crop and native genotypes, will be instrumental in mapping the regions of the genome involved. A mechanistic understanding of trade-offs in resource allocation is critical for identifying fundamental constraints on evolution and essential for continuing to improve domesticated crop production in a changing and increasingly stressful climate.

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The laboratory of L.H.R. studies the genetics of domestication, invasiveness, hybridization, and speciation, particularly in the genus *Helianthus*.

Data accessibility

Data deposited in the Dryad repository: doi:10.5061/dryad.gf647cc0.

DNA sequences: GenBank accessions AB199316, CX945586, AJ412547, CD848534, CD847616, CD847838, CD845611, CX947160, CD848175, CD847464, BU671886, BU671999, BU672086, BU671806, BU672004, BU671983, BU671885, QH_CA_Contig1047_1, QH_CA_Contig1784_4, QHN14D03, BG734517, BG734515, BG734514, CD847529 and CD847763.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Gene list of preliminary expression analysis.

Fig. S1 Plant growth and survival during drought stress.

Fig. S2 Expression analysis of the phosphatase 2C gene during drought response.

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