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Research Paper

Crop-wild sunflower hybridization can mediate weediness throughout growth-stress tolerance trade-offs

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ABSTRACT

Agricultural weeds are plants well-adapted to agricultural environments interfering directly and indirectly with crop production and causing important economic losses worldwide. Crop-wild hybridization is one of the main forces that have ruled weed evolution along with adaptation to agricultural (or benign) environments. Considering the competing demands for resources in any plant, adaptation to agricultural environments might result in an increase in growth but with lower tolerance to stress. In Argentina, most of the non-native *H. annuus* populations grow on roadsides, ditches, fences, hedgerows (ruderals), but there are also a few cases of *H. annuus* growing in agricultural field as weeds (agrestals). We asume that weediness of these agrestal biotypes came after crop hybridization as result of growth-stress tolerance trade-offs. Ruderal, agrestal (with evidence of crop introgression), and crop biotypes were contrasted under studies of drought and defoliation stresses, as well as for plant growth under non-stressful conditions and sequences of stress-related genes. The agrestal biotype was less tolerant to defoliation and drought than the ruderal biotype. Drought tolerance variation was largely explained by plant height rate (growth) and defoliation tolerance variation was mainly explained by biomass accumulation (resource allocation). Agrestal biotype sequences of two genes encoding transcription factors involved in stress response, *DREB2* and *NAC*, showed evidence of positive selection in the crop direction. Therefore, selection in the agricultural environment combined with crop hybridization driver the evolution of a well-adapted genetic variant of *H. annuus* with fast growth but reduced stress tolerance.

1. Introduction

Agricultural weeds impact crop production through direct competition for nutrients, moisture and light, and they cause the higher potential losses (34%) than animal pests and pathogens (losses of 18 and 16% respectively) (Heap, 2014; Oerke, 2006). Weeds can originate in three ways: 1) from wild populations, where selection acts on standing variation; 2) from hybrids between wild and crop taxa (exoferality), by which segregation produces extreme and intermediate phenotypes on which selection acts; and 3) from plants directly descended from crop varieties (endoferality) (Bagavathiannan and Acker, 2008; De Wet and Harlan, 1975; Ellstrand et al., 2010, 2013).

Crop-wild hybridization is the first step in the flow of novel crop alleles into native or wild populations (Ellstrand et al., 2013). After that, these novel crop alleles can be introgressed (permanent incorporation of genes from one set of differentiated populations into another) in the recipient population (Stewart et al., 2003). Hybridization can be responsible for the formation of new hybrid lineages (Abbott et al., 2013) or can cause the extinction of populations or species (Todesco et al., 2016). Crop-wild hybridization has played an important role in the adaptive evolution of weeds like weedy *Brassica rapa* (Warwick et al., 2008), weed beet (Arnaud et al., 2010), California wild radish (Campbell et al., 2006) and weedy rice (Xia et al., 2011). Despite some maladaptive traits steming from the crop (e.g. lack of seed dormancy and seed shattering), which may prevent introgression, under selection in agricultural environments (e.g. by herbicides or competition with crops) some crop traits, such as rapid growth, herbicide tolerance and early flowering, may enhance hybrid fitness,

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increasing the risk of introgression (Mercer et al., 2014, 2007). Moreover, under wild-like conditions (e.g. low water availability) some crop traits (e.g. head diameter, petiole length and leaf size) could be favored in crop-wild advanced generations (Owart et al., 2014).

Agriculture produces relatively stable environments, through practices such as conservation tillage, fertilization, herbicide use, monoculture or short-term rotations, which exert high selected pressure on the weedy community (Ghersa and Satorre, 2000; Neve et al., 2009; Poggio et al., 2013). If agricultural selection pressures are consistently maintained and there is a genetic basis for beneficial traits, then the weed populations found in the fields will have been enriched in alleles that allow the plants to survive under conditions associated with cropping practices (Owen et al., 2015). Some of the well-documented evolutionary changes observed in weeds are traits related to seed germination, leaf shape, flowering pattern, seed shattering, seed size and shape, and herbicide resistance. Also, stem and leaf growth rates, plant height, and branching habit contribute to the architecture of the plant canopy, and consequently determine the timing and effectiveness of the shading of weeds over crop plants (Ghersa and Satorre, 2000; Murphy and Lemerle, 2006; Vigueira et al., 2013). Furthermore, the plant strategy to adapt to different levels of productivity or disturbance, for instance, can result in a resource use trade-offs that ensure rapid growth at the expense of lower stress tolerance (Agrawal et al., 2015; Grime, 1977; Koehler et al., 2012; Liancourt et al., 2005). For example, in alien environments as compared to native environments, plants tend to have improved competitive abilities, but reduced resource allocation to herbivore defenses due to the absence of specialist herbivores (Blossey and Notzold, 1995). In agricultural environments, individuals of the weed *Ipomoea purpurea* resistant to glyphosate showed a trade-off between resistance and fitness, which was evident in the absence of the herbicide (Debban et al., 2015). According to this concept, in environments where rapid growth is positively selected as agroecosystems, a trade-off between growth and stress tolerance may constrain the evolution or maintenance of stress tolerance in agroecosystems, or environments where rapid growth is positive selected (Grime, 1977; Vila-Aiub et al., 2015). There is also strong evidence that genetic differences between populations subjected to selection in contrasting environments, play a central role in these growth-stress tolerance responses. In particular, some transcription factors play a key role producing phenotypic variation and hence are considered quite important in adaptation (Fan et al., 2014; Kane and Rieseberg, 2007; Mayrose et al., 2011). So, crop-wild hybrids may evolve into genetic variants that have adaptive traits inherited from the crop parent as well as traits that favor proliferation and persistence inherited from the wild parent, thereby creating a weedy population (Vigueira et al., 2013).

Helianthus annuus L. is native to North America but nowadays it is widely distributed throughout several continents with reports from North and South America, Europe, Africa, and Australia (Dry and Burdon, 1986; Heiser, 1954; Muller et al., 2009; Poverene et al., 2002; Ribeiro et al., 2010). Natural hybrids between wild and crop sunflower have been recorded in their native and non-native area (Arias and Rieseberg, 1994; Ureta et al., 2008) and crop genes may contribute to increasing weediness (Muller et al., 2011; Casquero et al., 2013). In the US, weedy *H. annuus* populations were not highly differentiated from geographically proximal wild *H. annuus* populations, indicating that the formers were more closely related to nearby wild populations than to other weedy populations, which may have evolved multiple times within *H. annuus* (Kane and Rieseberg, 2008). However, an analysis of the set of common weedy genes revealed parallel shifts in gene expression across different weedy populations, indicating parallel adaptation to agricultural conditions (Lai et al., 2008). In addition, when a wide range of weedy populations from different countries were analyzed and compared with native *H. annuus* populations, a trade-off between growth and stress tolerance was found (Koziol et al., 2012; Mayrose et al., 2011). The European weedy populations were genetically different from wild US populations and they exhibited an intermediate variability between wild US populations and crop varieties (Muller et al., 2011). Thus, weedy European populations probably originated from the introduction of crop-wild hybrids into the farmers' fields (Faure et al., 2002; Muller et al., 2011). In Argentina, *H. annuus* populations are mainly found in roadsides, ditches, fences, hedgerows and field margins (hereafter, ruderal biotype) (Poverene et al., 2009) and these are morphologically similar to native US populations (Cantamutto et al., 2010a; Garayalde et al., 2011). However, recently a few cases of *H. annuus* populations were found in an agricultural field as a weed (hereafter, agrestal biotype) (Casquero et al., 2013). A case in the Buenos Aires province reduced the sunflower crop yield by more than 50% with > 4 weeds m^{-2}. This weed showed strong evidence of crop introgression, with intermediate traits between the crop and ruderal sunflower. This population was more aggressive with the crop than the ruderal plants due to their faster initial growth (Casquero and Cantamutto, 2016). Based on our observations, it is highly likely that weediness of this agrestal biotype evolved after crop hybridization because invasive *H. annuus* populations, which have been naturalized in Argentina for at least 70 years (Poverene et al., 2002), were never before found in agricultural fields as weeds. Moreover, this biotype was established in a different environment (with different climatic, soil and human intervention features) and isolated from ruderal populations, making it an ideal scenario for studying the evolution of weediness under agricultural selection. Here, we focus on three groups of plants (hereafter biotypes): ruderal, agrestal and crop plants, which where contrasted under drought and defoliation stresses, plant growth under non-stressful conditions, and variability in sequences of stress-related genes. Our hypothesis is that the rapid adaptation of the agrestal biotype to agricultural environments came after crop-wild hybridization as result of growth-stress tolerance trade-offs.

2. Materials and methods

2.1. Plant material

Three biotypes of *H. annuus* were evaluated: ruderal (RUD), agrestal (AGR) and crop sunflower (CROP). The RUD biotype was represented by two *H. annuus* accessions collected in central Argentina (Cantamutto et al., 2010a): Río Cuarto (RCU; S 33°09, W 64°20) and Colonia Baron (BAR; S 36°10, W 63°52). The AGR biotype was represented by one *H. annuus* accession collected in central Argentina (BRW; S 38°16, W 60°07) (Fig. 1). The agrestal population showed clear evidence of crop introgression, like plants with absence of anthocyanin, apical branching, presence of a main head, head diameter greater than 6 cm, male sterility, and an oil content of 38.7% (Casquero et al., 2013). We only included one agrestal accession because it is the only stable population found since 2000. While we have found some wild plants in agricultural fields these could not be consider agrestal populations because there were in low quantity along field margins and/or "sporadic appearances" during these years. The CROP biotype was represented by two cultivars (Paraíso 104 CL from Nidera company, and VDH 487 from Advanta company) and two inbred lines (HA89 and B71). These cultivars and inbred lines were also considered as accessions.

2.2. Defoliation tolerance study

The defoliation tolerance study was designed to simulate biotic stress like that of defoliation caused by Lepidoptera. RUD, AGR and CROP biotypes were grown in a common garden and were irrigated. The rows were separated at 1.40 m and plants at 0.30 m apart in the row. At the R3 stage (Schneiter and Miller, 1981), four defoliation treatments (non-defoliated control, 33, 66 and 100% defoliation treatments) were applied to the all active leaves on the main stem of each plant (Muro et al., 2001). In defoliation treatments, a portion of each of the active leaves on the main stem were cut-off according to