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OPINION

Improving C_3 photosynthesis by exploiting natural genetic variation: *Hirschfeldia incana* as a model species

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Abstract

Despite research efforts toward improving crop photosynthetic energy conversion efficiencies over the past 40 years, photosynthetic efficiencies remain far from their theoretical maxima. A major challenge has been that plant photosynthesis is a complex process, controlled by many underlying genetic factors and highly dynamic in response to short-term environmental changes. Recent approaches to improving photosynthesis involved model-based identification of the bottlenecks in photosynthesis followed by their genetic modification (GM). While these approaches were successful and inspirational, their dependency on the use of GM techniques may restrict their implementation in some jurisdictions. We therefore suggest greater research focus on a different, yet complementary, approach to improving photosynthetic efficiency: the exploration and exploitation of natural genetic variation in photosynthesis. A substantial improvement in phenotyping and genotyping technology over the past decade has highlighted natural variation in photosynthetic sub-traits for crop and model species. However, a comprehensive understanding of all the factors responsible for photosynthetic limitations is still lacking. We therefore propose the use of high photosynthetic capacity species as models for the exploration of the physiological and genetic basis of high photosynthetic efficiency. While most high photosynthetic capacity species are not suitable as models due to complex genetics and evolutionary distance from crops, we have identified Brassicaceae species *Hirschfeldia incana* (L.) Lagr.-Foss as a promising candidate. In this perspective paper, we describe and advocate the use of *H. incana* as a model for the exploration of high maximum CO_2 assimilation rates (P_{max}) found in some C_3 species. We describe the basic biology and evolutionary history of the species and report preliminary data on its photosynthetic characteristics. Our findings suggest *H. incana* is an excellent model species for studies aiming at understanding natural genetic variation in photosynthetic efficiency.

Graham Taylor and Francesco Garassino contributed equally to the manuscript.

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KEYWORDS
high photosynthetic capacity, *Hirschfeldia incana*, model species, natural genetic variation, photosynthesis

1 | INTRODUCTION

Improving crop plant photosynthesis was first proposed about 40 years ago (Austin, 1989) but since then progress in realizing this goal has been limited. A major hurdle to progress has been the genetic and physiological complexity of photosynthesis and the difficulty of phenotyping for photosynthesis on a large scale. High-throughput photosynthetic phenotyping has been a challenge, creating one of the major bottlenecks to progress in breeding for better photosynthesis. Only in the last decade has the development of large-scale, high-throughput photosynthetic phenotyping technologies and advances in genotyping techniques allowed researchers to begin to understand the genetics underlying variation in photosynthetic traits (van Bezouw et al., 2019). Improvements in portable gas analysis systems now allow the routine measurement of assimilation in the field, while high-throughput phenotyping techniques—usually based on chlorophyll fluorescence—are now available for mass measurement. Here, "high-throughput" means taking a measurement of a photosynthetic property on several hundred plants per species multiple times per day rather than in the order of 10 to 100 plants per day which is typical when using portable gas analysis systems. These technological improvements in phenotyping have depended on our enhanced understanding of photosynthetic physiology, particularly in the use of chlorophyll fluorescence-derived parameters. More generally, there has also been an increase in larger-scale research into the genetics and scope of natural variation of photosynthesis of the kind needed to exploit natural variation in photosynthesis as a path to crop yield improvement (Dann & Leister, 2017; Flood et al., 2011; Theeuwen et al., 2022). Evidence of natural variation in photosynthetic sub-traits has now been reported for major staple crops such as wheat (Driever et al., 2014; Molero & Reynolds, 2020), rice (Acevedo-Siaca, Coe, Quick, et al., 2020; Acevedo-Siaca, Coe, Wang, et al., 2020; Gu et al., 2014; Lin et al., 2018; Wang et al., 2015), maize (Strigens et al., 2013), soybean (Burgess et al., 2020; Gilbert et al., 2011; Lopez et al., 2019), potato (Prinzenberg et al., 2018; Xu et al., 2021), and sorghum (Ortiz et al., 2017), as well as for the model species *Arabidopsis thaliana* (Prinzenberg et al., 2020; van Rooijen et al., 2015, 2017). Even for tree species, such as *Fagus sylvatica* (Aranda et al., 2014), whose photosynthetic properties are often less intensively studied than those of crop plants and model species, there is growing interest in the natural variation of their photosynthetic properties.

Photosynthesis is a complex process with many potential underlying genetic factors, and much remains to be discovered about the naturally occurring variability of the process (van Bezouw et al., 2019). In particular, photosynthesis has been extensively studied and understood as a steady-state process, while the kinetics of the photosynthetic response to a short-term change in the environment has been largely ignored (with some exceptions, e.g., Harbinson & Woodward, 1984; Pearcy, 1990). Recently, it has been more widely recognized that slow, limiting responses to environmental fluctuations over various timescales (typically >1 s) can result in sub-optimal CO_2 assimilation; this is particularly relevant for photosynthesis in the field, where it is now recognized that photosynthesis may only rarely be at steady-state (Lawson et al., 2012). The desire to increase future crop yield sustainably further complicates the challenge of yield improvement, so in addition to the obvious importance of photosynthetic light-use efficiency (LUE or quantum yield), the efficient use of water (Franks et al., 2015; Leakey et al., 2019; Richards et al., 1993), nitrogen (Hirel et al., 2007; Raun & Johnson, 1999; Swarbreck et al., 2019), phosphorous (Heuer et al., 2017; Veneklaas et al., 2012), and micronutrients (Dimkpa & Bindraban, 2016; Fageria et al., 2008; Monreal et al., 2016; Welch & Graham, 2002) for photosynthesis are increasingly recognized as important and genetically complex traits influencing crop production sustainability. Inevitably, traits connected to the efficiencies of nutrient and water use will come into greater focus as part of improving crop yields.

2 | PHOTOSYNTHETIC IMPROVEMENT POTENTIAL

As a process in higher plants, oxygenic photosynthesis is relatively inefficient (Cardona et al., 2018). This inefficiency is a major factor contributing to a realized yield productivity below the theoretical maximum in crop species (Zhu et al., 2010). The energy conversion efficiency of C_3 and C_4 pathways—including that of bioenergy crops—is only 2.4% (4.6% theoretical maximum) and 3.7% (6% theoretical maximum) based on the total incident solar radiation intercepted by a leaf canopy (Beadle & Long, 1985, 1995; Monteith, 1977; Piedade et al., 1991; Zhu et al., 2008). This photosynthetic

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inefficiency, while highlighting productivity gaps between realized and theoretical limits, also highlights the scope for future research toward improving photosynthesis potential. Given these gaps, it is increasingly argued that one of the best means available to allow the World food supply to meet the projected increases in global food demand is through the improvement of the photosynthetic performances of crop plants (Long et al., 2006; Murchie et al., 2009; Parry et al., 2011; Zhu et al., 2008, 2010). This will aid with meeting increased future demand for food implied by a global population increase alongside economic growth (Tilman et al., 2002), which remains a significant challenge; it is expected that crop yields will need to increase by up to 110% (Tilman et al., 2002; Zhu et al., 2010) and this should be achieved without further expansion of the world's land area dedicated to agricultural production otherwise we risk further loss of biodiversity.

Improving photosynthesis as a means to improve crop yield was first considered several decades ago (Gifford & Evans, 1981; Zelitch, 1975), but at that time the technical and scientific barriers to achieving this goal were insurmountable and the idea essentially lapsed. During the last two decades, however, the idea that improvements to photosynthesis will be our best biological option for significantly improving crop yields has come to the fore (Evans, 2013; Lawson et al., 2012; von Caemmerer & Evans, 2010; Zhu et al., 2010). In 2012, the "Realizing Increased Photosynthetic Efficiency" (ripe.illinois.edu) project initiative was formed, demonstrating an important commitment to this endeavor. While there has been some scientific debate on whether improved leaf-level C_3 photosynthesis would even translate into yield increases (Sinclair et al., 2004, 2019), evidence from CO_2 enrichment studies and genetic modification (GM) approaches support the idea that higher photosynthetic efficiency will translate to better yields (Long et al., 2006; Mitchell & Sheehy, 2006; Sheehy et al., 2008; von Caemmerer & Evans, 2010). Additionally, increased atmospheric CO_2 mole fraction or supplementary lighting can achieve improved photosynthesis in protected cultivation, such as greenhouses, resulting in higher yields. With field-grown crop plants, the scope for environmental modification to increase yield is limited and therefore significant crop yield gains are likely to be more dependent on genetic approaches. This implies that we need to find or create genetic diversity that is coupled with photosynthesis variation.

3 | HOW TO IMPROVE PHOTOSYNTHESIS

Recent approaches to improving photosynthesis have been guided by model-based identification of the bottlenecks in photosynthesis (Poolman et al., 2000; South et al., 2019; Zhu et al., 2010). These approaches have been successful and inspirational, but they depend on the use of GM techniques (Driever et al., 2017; Kromdijk et al., 2016; López-Calcagno et al., 2020; Simkin et al., 2015; South et al., 2019). The regulatory framework on the use of GM and similar novel plant breeding techniques (NPBT), however, can be restrictive and the commercial cultivation of NBPT-based cultivars has been highly limited in some jurisdictions such as the European Union. For these jurisdictions, an alternative to NPBT-based crop improvement is needed, such as conventional plant breeding exploiting naturally occurring trait variation. In addition to regulatory issues, GM (and similar) normally depends on an approach involving the intelligent creation, redesign, or modification (i.e., engineering) of photosynthetic traits. In general, this approach requires a thorough knowledge of the physiological or developmental pathway targeted for improvement. The NPBT route to improvement is therefore likely to be limited because many photosynthetic traits are not yet sufficiently understood for NPBT approaches to be able to be applied successfully.

Alongside GM research, we therefore suggest greater research focus on a different, yet complementary, approach to improving photosynthetic efficiency: the exploration and exploitation of natural genetic variation in C_3 photosynthesis. The natural, intraspecies variation for photosynthetic traits in species that can be crossed with crop plants could be used to improve crop photosynthesis via conventional plant breeding routes, resulting in better photosynthetic assimilation rates, and, ultimately, a higher yield potential (Lawson et al., 2012; Zhu et al., 2010). Making use of this variation in conventional breeding is more efficient if the genes or quantitative trait loci (QTLs) underpinning the variation for photosynthetic traits have been identified. Such genes can be further studied to understand the biology explaining the variation and can become a target for marker-assisted breeding, gene editing or GM approaches. On a broader scale, the physiological diversity of photosynthesis within plants and algae represents a collection of templates or models for particular traits, such as a very high maximum CO_2 assimilation rate or exceptional qE (an important photoprotective mechanism acting in Photosystem II, *PSII*). Unraveling the physiological and genetic bases of exceptional traits is a route to building more energy-efficient photosynthesis, and assists in understanding the physiological and genetic limits of genotypes with phenotypic deficiencies, such as normal maximum CO_2 assimilation rates or normal qE.

4 | LEAVES, PHOTOSYNTHESIS, AND TRAITS

The leaf is the organizational level of the plant at which the properties, or traits, of photosynthesis, and particularly the

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environmental dependency of these traits, are commonly assessed. These leaf-level properties arise from an underlying, highly coordinated developmental process that builds and maintains the photosynthetic machine embedded in the leaf mesophyll cells. This process also gives rise to larger-scale leaf-structural properties that provide the diffusive pathways for efficient gas exchange, and the vascular tissues essential for the transport of water, nutrients, and assimilates. Photosynthesis is a highly flexible process that is strongly influenced in the short to long term by the environment in ways and extents that depend on genetic differences between individuals and species. This genetic control of photosynthetic traits, however, is generally not understood despite the thoroughness with which variation in photosynthetic traits is sometimes understood at the physiological level.

Maximum CO_2 assimilation rate (P_{max}) is a good example of a leaf-level photosynthetic trait. In higher plants, P_{max} depends on a range of other sub-traits, such as high rates of electron transport and photosynthetic metabolism, and high stomatal and mesophyll conductances for CO_2 diffusion. These broad sub-traits can themselves be further refined into yet more specific sub-traits and the scaling from the cellular level of proteins and membranes to the whole leaf is reasonably well understood. However, the genetic basis for P_{max}—its *genetic algorithm*—is not known despite this trait being relatively well understood from a photosynthetic physiology perspective. Increasing P_{max} through marker-assisted selection breeding, therefore, is not currently feasible (although breeding based on phenotype selection alone is possible). Identifying the genes responsible for variation in P_{max} would make marker-assisted breeding possible, and would also make available targets for GM or gene editing approaches.

Apart from its value in breeding, understanding the genetic basis of variation in photosynthetic traits is also a way to understand the developmental process that builds the photosynthetic machinery and delivers the diverse range of photosynthetic phenotypes encountered in the natural world. The evolution of photosynthetic traits is recorded in these—as yet largely unknown—genetic underpinnings of photosynthetic variation. Understanding the genetic basis of natural variation in plant photosynthesis, therefore would also give us access to the evolutionary history of photosynthetic variation in plants, and to the adaptive mechanisms that allow plants to successfully occupy specific environmental niches (Flood, 2019; Flood et al., 2011). This knowledge is of particular significance for agriculture given the extent and rate of environmental change and the rapidly growing human population that demands crop plant phenotypes to be creatively adapted.

Technological advances in photosynthetic measurement equipment and techniques have assisted in our understanding of photosynthetic physiology and its variation by allowing for highly integrated and detailed measurement of photosynthesis—including both deep-phenotyping and high-throughput phenotyping. Deep phenotyping makes possible the analysis and identification of photosynthetic bottlenecks under diverse environmental/experimental conditions through the combined application of a range of destructive and non-destructive measurement techniques. The same chlorophyll fluorescence measurements that are often used in deep-phenotyping strategies are typically used in high-throughput phenotyping for photosynthetic traits (Flood et al., 2016, 2020; Furbank & Tester, 2011). A high-throughput phenotyping approach can therefore provide the extensive data needed for the identification of the QTLs contributing to variation in trait properties. These phenotyping approaches, together with advances in genomics, make it possible to genetically compare related species with differing photosynthetic traits, and to understand the changes leading to different photosynthetic phenotypes. What remains is the identification of model species on which to effectively analyze complex physiological traits like photosynthesis based on trait function and genetics.

5 | PHOTOSYNTHETIC LIMITATION

The photosynthesis-irradiance response provides a useful handle to understand and quantify multiple limitations acting on a leaf. When crops and other plant species are exposed to increasing irradiances, leaf-level photosynthetic rates tend to become light-saturated well below the typical, full-sunlight levels of 2000 $\mu mol\, m^{-2}\, s^{-1}$ or more encountered under natural conditions (Figure 1) (Gitelson et al., 2015; Gu et al., 2017; Monneveux et al., 2003; Murchie et al., 1999; Turner et al., 2003). At strictly light-limiting irradiances, photosynthetic LUE is maximal; LUE is defined here as the ratio between gross photosynthesis (A_{gross}, $\mu mol\, m^{-2}\, s^{-1}$) and the absorbed or incident photosynthetic photon flux density ($\mu mol\, m^{-2}\, s^{-1}$). Maximum, light-limited, photosynthetic efficiency is a complex trait and varies, inter alia, with the wavelengths of light used, photosynthetic metabolism (especially the activity of photorespiration (Ehleringer & Björkman, 1977)), state-transitions the presence of non-photosynthetic pigments (Hogewoning et al., 2012), and photodamage to photosystems I and II (e.g., Kao & Forseth, 1992; Sonoike, 2011).

At irradiances above the strictly light-limited range (up to about 100–200 $\mu mol\, m^{-2}\, s^{-1}$ for a typical C_3 crop plant) other physiological and metabolic limitations become significant. These limitations result in a decline in overall LUE from the light-limited maximum, and

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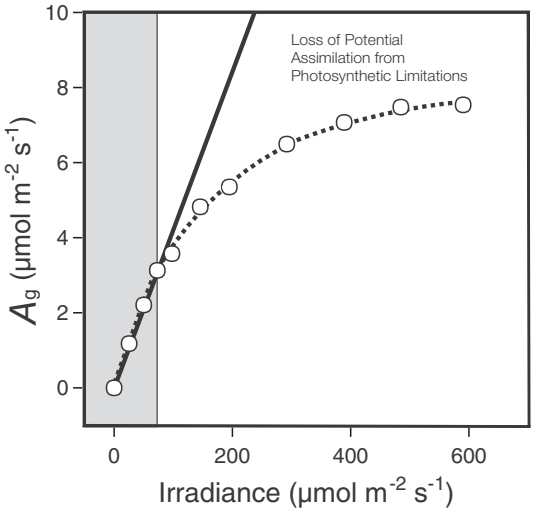


FIGURE 1 A non-rectangular hyperbola light response curve of gross photosynthetic rate (A_g) with increasing irradiance for a typical, low-light (200 μmol m^{-2} s^{-1}) adapted C_3 plant leaf. The range of irradiances shaded in gray is where photosynthesis is significantly light-limited. The region between the extrapolation of the light-limited phase (solid line) and the assimilation response (dotted dash response curve fit estimate) is where photosynthetic efficiency declines. Understanding this natural variation in efficiency loss at saturating irradiances is key to providing research handles for future genetic research.

ultimately in the light saturation of photosynthesis. An implication of this is that in the absence of these constraints the photosynthesis–irradiance relationship would be linear with a gradient equal to the light-limited slope of the photosynthesis–irradiance relationship (Figure 1). Although the underlying biochemical components and mechanisms of achieving photosynthesis are highly conserved among C_3 species, the light-saturated assimilation rates arising from the loss of photosynthetic LUE with increasing irradiance can vary considerably among genotypes and species. While a higher P_{max} arises mainly from the ability to sustain a higher LUE at very high irradiances, this high LUE at high, near light saturating irradiances will be associated with higher light-use efficiencies and therefore assimilation rates at lower irradiances above the strictly light-limited region of the photosynthetic light-response curve (Harbinson & Yin, 2017). If the high P_{max}, high LUE of high light-adapted leaves is paralleled by a relatively high P_{max}, and thus a still high LUE, following adaptation to lower irradiances found deeper in the canopy, the high P_{max} phenotype will benefit not only the assimilation in those leaves exposed to a full irradiance but also those in more shaded parts of the canopy.

A sustained high rate of photosynthesis, which implies a high LUE for photosynthesis at high irradiances, requires that all of the supply and demand side processes can support high fluxes. A high light-saturated C_3 photosynthetic rate, in terms of a CO_2 fixation rate, is usually around 30 μmol m^{-2} s^{-1}, while C_4 leaves typically achieve rates of 30–60 μmol m^{-2} s^{-1}. Some C_3 leaves are capable of assimilation rates of up to 65 μmol m^{-2} s^{-1}, which is better than most C_4 crops, but how these leaves achieve such high assimilation rates in terms of either their physiology or genetics is not clear. The potential flux limitations to steady-state C_3 leaf photosynthesis are numerous but can be summarized into four main groups of processes. Assuming that the product of photosynthesis is mesophyll cell carbohydrate, these four groups can be further split into three supply-side processes and one demand-side process. The main supply-side processes are the diffusive transport of CO_2 via the stomata and mesophyll conductive paths to the site of fixation; the light reactions with electron transport providing the reductant (NADPH) and ATP for the metabolic processes of assimilation; and photosynthetic metabolism which fixes CO_2 and produces carbohydrate. The demand-side process is the transport of carbohydrates to the sink tissues and their use by the sinks. Leaves with a high photosynthetic capacity must be able to develop high conductances for the gaseous diffusion of CO_2, a high metabolic capacity, and high rates of electron transport on the supply side, along with high carbohydrate transport and sink activity to allow for sustained high photosynthetic LUE at high irradiances. High photosynthetic capacity exemplar leaves offer models within which to explore the physiological and genetic basis of high P_{max}.

6 | NATURALLY OCCURRING HIGH-PHOTOSYNTHETIC CAPACITY SPECIES

A. thaliana became the model C_3 plant species for plant research over the last 40 years mainly because it has many desirable characteristics for experimentation. These include a short regeneration time, a small plant size, a limited need for growth facilities, prolific self-fertilization, and an increasingly multidisciplinary research environment (Koornneef & Meinke, 2010). Despite the well-documented merits and benefits of research focused on *A. thaliana*, there have been concerns that the focus on *A. thaliana* may lose impetus as plant science research funding and community interest in the species decline (Koornneef & Meinke, 2010). Wild-type *A. thaliana* is of limited applicability in research directed toward enhancing C_3 photosynthesis by increased photosynthetic capacity because of its unexceptional photosynthetic capacity; light-saturated assimilation rates of 6.5–12 μmol m^{-2} s^{-1} have been reported for *A. thaliana* (Kaiser et al., 2016; Tanaka et al., 2013) grown at the low growth irradiances

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($100–200\,\mu mol\,m^{-2}\,s^{-1}$) typically used for this species under controlled environment conditions. When grown at high irradiances ($1800\,\mu mol\,m^{-2}\,s^{-1}$) *A. thaliana* can achieve assimilation rates of $30\,\mu mol\,m^{-2}\,s^{-1}$ (Garassino et al., 2022), which while high when compared to results obtained from this species under commonly used irradiances, are not exceptional compared to some other C_3 species. Evidently, although *A. thaliana* is an excellent and well-researched general C_3 plant model, it lacks the photosynthetic properties that would make it a candidate model for exceptional P_{max}.

Among C_3 plants, sustained high LUE at high irradiances, resulting in a P_{max} over $40\,\mu mol\,m^{-2}\,s^{-1}$, have only been reported for a few species. The majority of these are annuals endemic to desert environments of the South-Western USA, where a high P_{max} is attributed to an adaptation to very short growing seasons in a high-light environment (Seemann et al., 1980). Nobel (1991) calculated a theoretical maximum net photosynthetic rate for C_3 plants of about $55\,\mu mol\,m^{-2}\,s^{-1}$ at an irradiance of $2000\,\mu mol\,m^{-2}\,s^{-1}$. Werk et al. (1983), however, reported the highest photosynthetic capacity for a C_3 plant of $65\,\mu mol\,m^{-2}\,s^{-1}$ by *Palafoxia linearis*, a winter desert annual, grown in a controlled environment. The C_3 winter desert annuals *Camissonia claviformis* and *Camissonia brevipes* (now part of the Chylismia genus) achieve an assimilation rate of about $60\,\mu mol\,m^{-2}\,s^{-1}$ at an irradiance of $2000\,\mu mol\,m^{-2}\,s^{-1}$ (Ehleringer et al., 1979; Longstreth et al., 1980; Seemann et al., 1980). Other C_3 species have been reported to have high assimilation values of $42–55\,\mu mol\,m^{-2}\,s^{-1}$ (Nobel, 1991) at an irradiance of $2000\,\mu mol\,m^{-2}\,s^{-1}$; even at this high irradiance, these assimilation rates were not fully light-saturated. Light-saturated leaf photosynthetic assimilation rates for a typical cereal crop, for example, wheat, are only $20–30\,\mu mol\,m^{-2}\,s^{-1}$ under physiologically favorable conditions. The high photosynthetic capacities of the C_3 desert annual species referred to are comparable to, and even exceed, those of many C_4 crop species, which typically produce biomass more efficiently than C_3 crop plants on an intercepted light basis (von Caemmerer & Evans, 2010). The C_4 species *Amaranthus palmeri* (considered a problem weed in some regions), however, can achieve an assimilation rate of nearly $80\,\mu mol\,m^{-2}\,s^{-1}$ when measured at an irradiance of $2000\,\mu mol\,m^{-2}\,s^{-1}$, an optimal temperature of 42°C, and at natural ambient CO_2 concentrations (Ehleringer, 1983)—the highest known photosynthetic assimilation rate for any plant species (although this claim must be tempered by the lack of any extensive, focused effort to find better performing species). Despite the high assimilation rate potential revealed by these C_3 and C_4 species, many of the high photosynthetic capacity species mentioned here would not qualify as good experimental plant models. Good model plants demand simple genetics and a close genetic distance from major crop plant species or another genetically well-researched species; for example, neither *A. palmeri* (Amaranthaceae) nor *Camissionia*/*Chylismia* species (Onagraceae), share close phylogenetic relations with major commercial crops. Furthermore, they have scarce curated germplasm and are poorly studied genetically. To understand the genetic and physiological basis of high LUE at high irradiances, a model species that has high assimilation rates at high irradiance and is genetically close to crop or model plant species is therefore needed. *Hirschfeldia incana* appears to offer that special combination of physiological and genetic properties that support its candidacy as a model species. We need to recognize however that while *H. incana* may be the most convenient, suitable model species for the very high photosynthetic rate phenomenon, we should continue comparing this with other species that also have very high photosynthetic rates; there may be different routes to the same end.

7 | *HIRSCHFELDIA INCANA*

H. incana (L.) Lagr.-Foss. (Figure 2) is a nitrophilous and thermophilous winter annual or biennial C_3 species. It is the sole member of a monophyletic genus and is native to the Mediterranean basin and the Irano-Turanian floristic region (Manafzadeh et al., 2017). Due to introductions elsewhere, the distribution of *H. incana* extends to cool and warm-temperate climate areas globally (Siemens, 2011), with wild populations concentrated in the sub-tropical to temperate regions of the world (Figure 3).

In these regions, *H. incana* occurs as a pioneer species in colonies often of only a few individuals or as solitary plants on disturbed sites such as roadsides and ditches (DiTomaso & Healy, 2007). It shows rapid, sustained growth over a short period, and is commonly seen as a weedy, invasive species (Lee et al., 2004). *H. incana* is a prolific bearer of seeds, which are contained in numerous siliques. About 3–5 seeds per locule develop along many long racemes (DiTomaso et al., 2013). Seeds obtained from a wild population at Oued El Himer in Eastern Morocco were 1.16 ± 0.05 mm in length and 0.86 ± 0.03 mm in width ($n = 10$, unpublished observation), and seeds are generally highly viable at maturation (Gresta et al., 2010), germinating rapidly within a few days without requiring any seed pre-treatment (unpublished observation). Rapid germination was reported at 25–30°C in light conditions and 20–25°C in darkness by Gresta et al. (2010), while we have found high germination rates in fresh seed material exceeding 90% at 20°C in darkness and 23°C in light. Seeds of *H. incana* store well, consistent with its classification as

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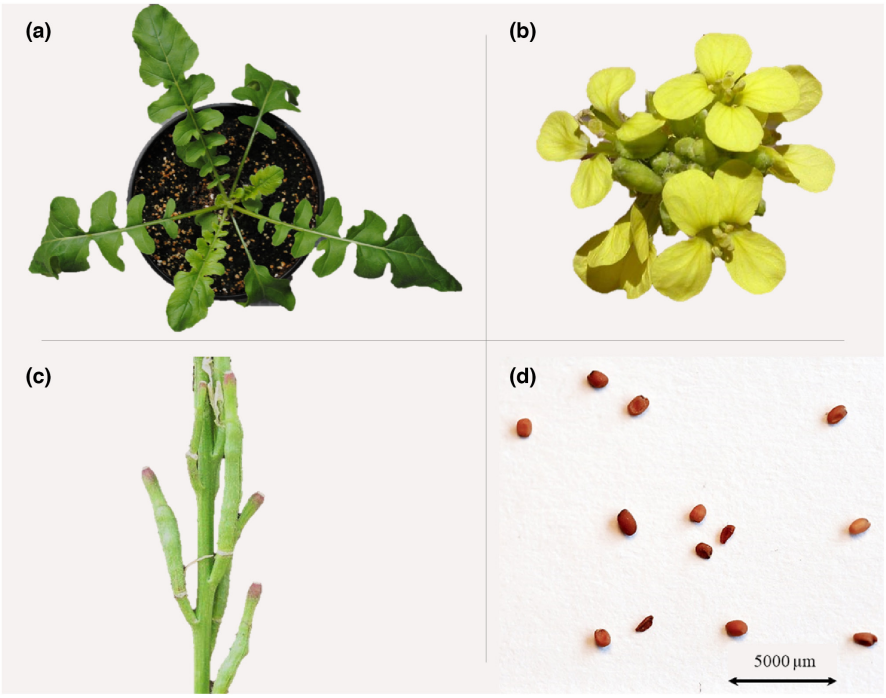


FIGURE 2 The morphology of *Hirschfeldia incana*. (a) Top view of a 4 weeks-old plant grown under artificial lighting in a controlled indoor environment, (b) typical inflorescence, (c) maturing siliques, and (d) micrograph of ripe seeds.



FIGURE 3 The global distribution of wild populations reported for *Hirschfeldia incana* (various sources, see Quiroz (2015)). Orange markers indicate approximate locations where at least one individual has been reported in the wild.

a weedy species; when stored under non-ideal seed storage conditions of 23°C and at a relative humidity of 50%, we found most *H. incana* seeds remained viable for more than 5 years. Under favorable growth conditions, *H. incana* has a relatively short reproductive cycle, completing germination to flowering in about 5–8 weeks. The maturation of the first seed set occurs approximately 3–4 weeks after flowering. Although *H. incana* is considered self-incompatible

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due to protogyny (Al-Shehbaz, 1977), self-fertilization has been reported (Lee et al., 2004) and self-fertilized progeny can be produced with hand pollination. Seedlings of *H. incana* establish and grow well in pots under neutral white LEDs or fluorescent lighting commonly used in growth chambers, and they thrive under warm conditions (Chronopoulos et al., 2005).

As a member of the Brassicaceae (tribe Brassiceae), *H. incana* is a close relative of economically important vegetable and oil-seed crop species (FAO, 2022), including *Brassica rapa, Brassica oleracea*, *Brassica napus*, *Brassica nigra*, and *Camelina sativa* (Arias & Pires, 2012; Huang et al., 2016; Warwick & Black, 1991). *H. incana*, therefore, belongs to one of the most well-studied plant families with regard to phylogeny, physiology, genetics, and genomics (Al-Shehbaz, 2012; Arias & Pires, 2012; Franzke et al., 2011; Nikolov & Tsiantis, 2017), and which has been subject to in-depth comparative genomics studies (Koenig & Weigel, 2015; Schranz et al., 2006; van den Bergh et al., 2016). The fairly close phylogenetic relationship shared between *H. incana* and *A. thaliana* makes it feasible to swap tools and knowledge developed for either species. *H. incana* has a somatic chromosome number of $2n = 14$ (Anderson & Warwick, 1999; Rollins, 1981), and an estimated genome size of about 450 Mb (Garassino et al., 2022), which modern genomics technologies make accessible. The evolutionary history that *H. incana* shares with its relatives involve a series of ancient whole genome duplication and triplication events interspersed with diploidization events (Franzke et al., 2011). This complex evolutionary history is believed to have resulted in a great potential for the emergence of new genetic functions or the preferential retention of favorable genes in Brassicaceae species exposed to different environmental constraints, thus resulting in their adaptation to these environments (Cheng et al., 2014; Qi et al., 2021; Zhang et al., 2021). The genetic blueprint for the evolution of high assimilation rate characteristics of *H. incana* may have arisen from the preferential retention or the evolution of specific photosynthetic efficiency-related genes because of its evolution in dry, hot, and sunny climates with ephemeral, low competition habitats.

The widespread distribution of *H. incana*, combined with its large seed production and ease of germination and growth, allows for the easy building of diverse panels, as demonstrated by the fact that without any targeted collection campaign our laboratory was able to obtain more than 30 accessions from three continents. Intercrossing between these accessions proved to be easy, and the resulting progeny can easily be propagated, implying that the construction of mapping populations in *H. incana* is a feasible task. Hybridization of *H. incana* has been shown to be possible with commercial Brassica crops such as *B. napus* (Darmency & Fleury, 2000; Lefol et al., 1995; Siemens, 2002) and to some extent *B. oleracea* (Quiros et al., 1988), on other Brassicaceae species such as *B. nigra* (Quiros et al., 1988; Salisbury, 1991) and *B. carinata* (Mohanty et al., 2007), as well as with very close relatives such as *Erucastrum elatum* and *Erucastrum virgatum*, based on our own experience. Thus, the introgression of genes underlying the photosynthetic efficiency of *H. incana* appears possible, with the best candidate for introgression being *B. napus* (Devos et al., 2009).

8 | PHOTOSYNTHETIC CHARACTERISTICS OF *H. INCANA*

H. incana exhibits exceptional C_3 photosynthetic characteristics (Canvin et al., 1980). A photosynthetic capacity of 45 to 55 $\mu mol\ m^{-2}\ s^{-1}$ was attained by young, fully expanded leaves when measured under 21% O_2, 400 ppm CO_2 and 70% relative humidity (Figure 4). Although these rates are not as high as the highest assimilation rates recorded for *Camissionia*/*Chylismia* species or *Palafoxia linearis* (Ehleringer et al., 1979; Longstreth et al., 1980; Seemann et al., 1980; Werk et al., 1983), *H. incana* has the advantage of comparatively simple genetics and being a member of the Brassicaceae family. Although high photosynthetic capacity was recently reported for another species from the Brassicaceae family, *B. rapa* (Taylor et al., 2020), *H. incana* currently represents the upper limit for what might

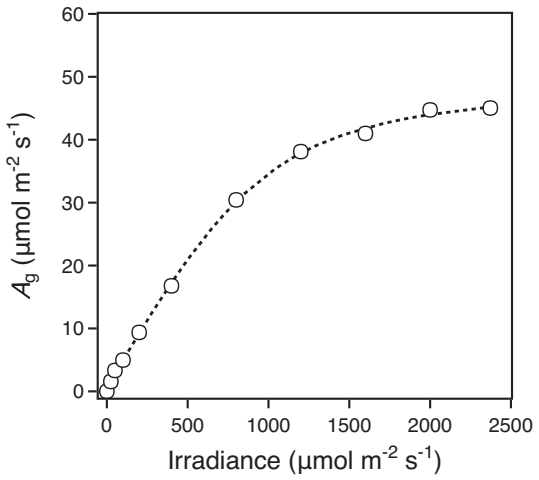


FIGURE 4 Gross photosynthetic assimilation rate (A_g) was measured at 21% O_2, 400 ppm CO_2 and 70% relative humidity for a representative, young, fully expanded *Hirschfeldia incana* leaf grown under 1800 $\mu mol\ m^{-2}\ s^{-1}$ PAR irradiance. Markers (○) denote measured data obtained using a 660 nm spectral peak actinic light source. The dotted line shown passing through markers is a fit estimate of the response using a non-rectangular hyperbolic equation (Acock et al., 1976; Thornley, 1976).

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be the highest achievable photosynthetic capacity in the family. While in an ideal scenario one would like to have a model species with large intra-species variation for P_{max} under similar growth conditions, this has not been reported for any plant species to date. Nevertheless, a degree of natural variation in photosynthetic capacity was already observed for *H. incana* (Garassino et al., 2022), and the aforementioned widespread distribution of the species across geographical locations and climates suggests that more variation can be easily accessed, potentially allowing for the reporting of even higher photosynthetic capacity.

The rate constant for linear electron transport (measured using the dark relaxation of the 820 nm absorbance change as a measure of kinetic limitation imposed by the photosynthetic electron transport chain (Baker et al., 2007)) generally had a $t_{1/2}$ of 1.7–2.5 ms (equivalent to a rate constant of 272–230 s^{-1})—significantly higher than values obtained, for example, pea and spinach leaves (Harbinson & Hedley, 1989; Schreiber et al., 1989). The proton efflux rate constant via the ATPase (measured via the 520 nm light-induced absorbance change (Baker et al., 2007)) in *H. incana* leaves is nearly constant at 80 s^{-1} in the irradiance range 1250–2400 μmol m^{-2} s^{-1}.

The decline of Φ_{PSII} and Φ_{PSI} with increasing irradiance was found to be modest for *H. incana*, decreasing to only 0.43 (Φ_{PSII}) and 0.61 (Φ_{PSI}) at an irradiance of 2400 μmol m^{-2} s^{-1} (Figure 5). Similarly, the decline of F_v/F_m and F_v'/F_m' with irradiance was modest up to a saturating irradiance of 2400 μmol m^{-2} s^{-1} (Figure 6). The relationship between Φ_{PSII} and Φ_{PSI} with increasing irradiance was also relatively linear, a finding consistent with results obtained from other species (Figure 7).

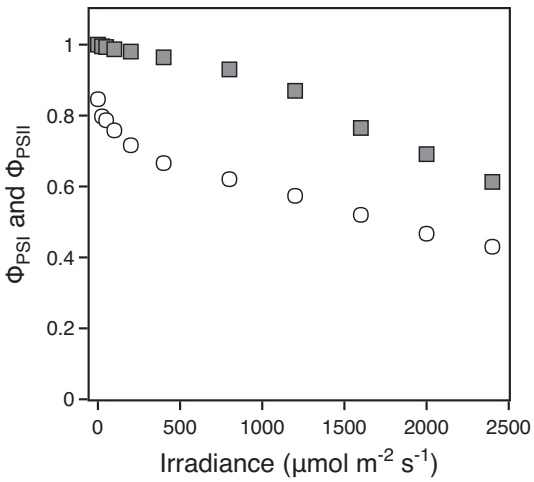


FIGURE 5 Φ_{PSI} (■) and Φ_{PSII} (○) with increasing irradiance measured for a representative, young, fully expanded *Hirschfeldia incana* leaf grown under 1800 μmol m^{-2} s^{-1} PAR irradiance. The measurement was made at 21% O_2 and 400 ppm CO_2 using a 660 nm spectral peak chlorophyll fluorescence excitation.

Therefore, the photosynthetic apparatus in *H. incana* leaves is remarkably robust to high irradiances, exhibiting little to no physiological stress or photoinhibition at measurement irradiances of up to 2400 μmol m^{-2} s^{-1}—levels higher than could be expected on a cloudless day at noon in the Equator during an equinox (~2200 μmol m^{-2} s^{-1}) or for horizontal locations at 37°N during a summer solstice solar noon (~2130 μmol m^{-2} s^{-1}) (Ritchie, 2010). In

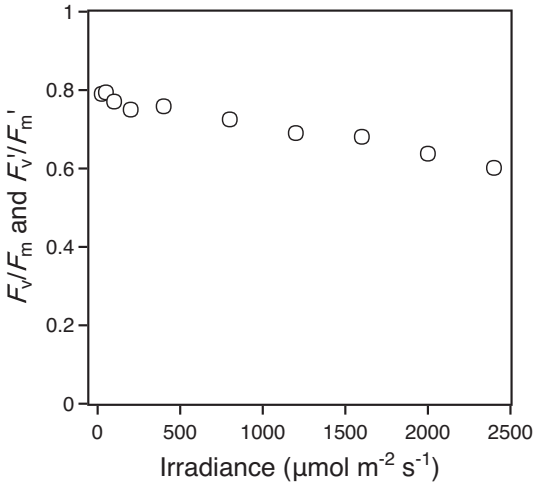


FIGURE 6 F_v/F_m (single dark-adapted measurement), and F_v'/F_m' (light-adapted measurements) with increasing irradiance, measured for a representative, young, fully expanded *Hirschfeldia incana* leaf grown under 1800 μmol m^{-2} s^{-1} PAR irradiance. The measurement was made at 21% O_2 and 400 ppm CO_2 using a 660 nm spectral peak chlorophyll fluorescence excitation.

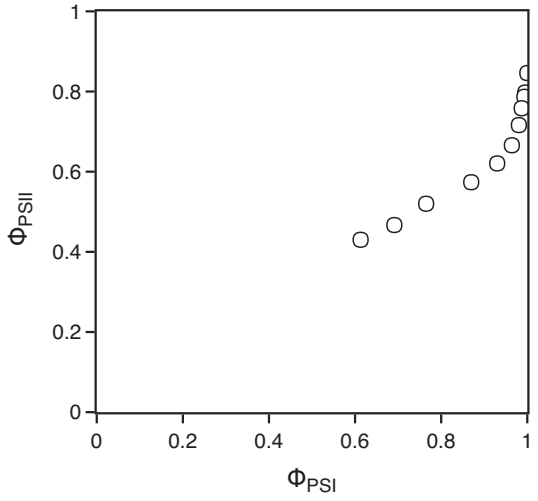


FIGURE 7 Operating efficiencies of photosystems PSI (Φ_{PSI}) and PSII (Φ_{PSII}) with increasing irradiance, measured for a representative, young, fully expanded *Hirschfeldia incana* leaf grown under 1800 μmol photon m^{-2} s^{-1} PAR irradiance. The measurement was made at 21% O_2 and 400 ppm CO_2 using a 660 nm spectral peak excitation of PSII and 820 nm spectral peak excitation of PSI.

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our hands, juvenile *H. incana* plants could be exposed to 2400 µmol m^{-2} s^{-1} for up to 12 h a day or 2000 µmol m^{-2} s^{-1} for 16 h without detectable signs of physiological stress or a decrease in the dark-adapted F_v/F_m values. These exceptional high light performance and tolerance characteristics of *H. incana* support its candidacy as a model C_3 species in research focused on improving C_3 crop photosynthesis.

9 | CONCLUDING THOUGHTS

A. thaliana did and continues to play a pivotal role as a model plant species in wide-ranging ecophysiological, photosynthesis, and genetic research. However, for research directed at bettering our understanding of the genetic, anatomical, and physiological adaptations required for sustained high rates of photosynthesis in a C_3 plant, *H. incana* can become a valuable complementary plant model. It is capable of withstanding, growing under, and photosynthetically making efficient use of very high irradiances. The diploid genetics and position within the Brassicaceae make *H. incana* genetically simple and allow the wealth of genomic information that exists for this family to be leveraged. In particular, the broad depth of genetic and physiological knowledge, and the techniques, available for *A. thaliana*, another Brassicaceae species, further strengthen the case for using *H incana* as a high-light tolerant, high assimilation rate model species. In our hands the plant has also proven easy to grow in both greenhouses and growth cabinets—the greatest challenge was increasing fertilization and watering regimes to meet the high demands of the plant. At least in Western Europe, the seed is available from naturalized plant populations, although currently no diversity panels, or similar, exist for the species. We expect that more species with exceptional photosynthesis traits like *H. incana* will be established as models, which can collectively aid our understanding of how these traits function, and how they emerge from genetic specialization.

AUTHOR CONTRIBUTIONS

Graham Taylor, Francesco Garassino, and Jeremy Harbinson conceptualized the manuscript. Graham Taylor and Francesco Garassino drafted the manuscript using data derived from Graham Taylor; Graham Taylor designed the figures. Jeremy Harbinson and Mark G. M. Aarts revised and finalized the manuscript content and outline.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The data used in the preparation of this manuscript will be made available upon request.

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