Pleiotropy in Triazine-Resistant *Brassica napus:* Leaf and Environmental Influences on Photosynthetic Regulation

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Since the first discovery of *s*-triazine-resistance (R) in higher plants, the altered D-1 protein product of the *psb*A gene has been regarded as less photosynthetically efficient in those R biotypes of a species. Decreases in electron transport function in the chloroplast have been believed to be the cause of decreased carbon assimilation rates and plant productivity in many reports. What is less clear in the literature is whether this change in D-1 structure and electron transport function directly modifies whole-leaf photosynthesis and plant productivity or only indirectly influences these functions. The dynamic nature of these responses have led several to conclude that the primary effect of R is complex, involves more than one aspect of photosynthesis, and can be mitigated by other processes in the system. Electron transport limitations are only one possible regulatory point in the photosynthetic pathway leading from light-harvesting and the photolysis of water, through ribulose bisphosphate carboxylase/oxygenase, to sucrose biosynthesis and utilization. Herein we discuss this complex issue, arguing that D-1 function can't be evaluated in isolation from the leaf, the organism, and possibly from the community response. Carbon assimilation in R and the susceptible wild type (S) is a function of several interacting factors. These include the pleiotropic effects resulting from the *psb*A mutation (the dynamic reorganization of the R chloroplast) interacting with other regulatory components of photosynthesis, microenvironmental conditions, and time (including ontogeny and time of day). Previous work in our laboratory indicated a consistant, differential, pattern of Chl *a* fluorescence, carbon assimilation, leaf temperature, and stomatal function between S and R *Brassica napus* over the course of a diurnal light period and with ontogeny, *i.e.* R is a chronomutant. Dekker and Sharkey have shown that the primary limitation to photosynthesis changes with changes in leaf temperature, and that electron transport limitations in R may be significant only at higher temperatures. The recognized R plants interact with the environment in a different way than does S. Under environmental conditions highly favorable to plant growth, S often has an advantage over R. Under certain less favorable conditions to plant growth, stressful conditions, R can be at an advantage over S. These conditions may have been cool (or hot), low light conditions interacting with other biochemical and diurnal plant factors early and late in the photoperiod, as well as more complex physiological conditions late in the plant's development. It can be envisioned that there were environmental conditions in the absence of *s*-triazine-herbicides in which R had an adaptive advantage over the more numerous S individuals in a population of a species. Under certain conditions R might have exploited a photosynthetic niche under-utilized by S.

Introduction

s-Triazine-resistance (R) in higher plants was first discovered in *Senecio vulgaris* in 1969 [1]. Subsequent research has shown R is due to a single base pair mutation to the *psb*A chloroplast gene [2]. The codon 264 change in the *psb*A gene causes a change in its product in *Amaranthus hybridus,* the D-1 protein, a key functional element in PS II electron transport [2]. *s*-Triazine-resistant plants have been shown to have a decreased quantum efficiency of CO_2 assimilation compared to *s*-triazine-susceptible plants (S) [3]; and have been generally regarded as less fit than S plants [4]. This decreased efficiency is credited to an altered redox state of PS II quinone acceptors and a shift in the equilibrium constant between Q_A^- and Q_B in favor of Q_A^- [5].

What is less clear in the literature is whether this change in D-1 structure and electron transport function directly modifies whole-leaf photosynthesis and plant productivity or only indirectly influences these functions [7]. The dynamic nature of these responses have led several to conclude that the primary effect of R is complex, involves more than one aspect of photosynthesis, and can be mit-

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igated by other processes in the system [7–9]. For example, it has been pointed out that decreased Q_A^- to Q_B electron transport in R is more rapid than the normally rate-limiting oxidation of plastoquinol [10, 11], while others studies indicate this step may be rate limiting [12]. Herein we discuss this complex issue, arguing that D-1 function can't be evaluated in isolation from the leaf, the organism, and possibly from the community response. Carbon assimilation in R and the susceptible wild type (S) is a function of several interacting factors. These include the pleiotropic effects resulting from the *psb*A mutation (the dynamic reorganization of the R chloroplast) interacting with other regulatory components of photosynthesis, microenvironmental and microhabitat conditions, and time (including ontogeny and time of day). It is also argued herein that electron transport sometimes indirectly regulates carbon assimilation in R, in other instances it has no apparent effect on regulation.

Discussion

Carbon assimilation in R and S

Several studies have shown lower photosynthetic carbon assimilation rates (A) in R *Amaranthus hybridus* [13] and *Senecio vulgaris* [3, 11] relative to S. Beversdorf *et al.* [14] found lower R whole plant yields in field evaluations of *Brassica napus*. Additionally, van Oorschot and van Leeuwen [15] found A in S *Amaranthus retroflexus* was greater than that in R; while R and S carbon assimilation were comparable in *Chenopodium album, Polygonum lapathifolium, Poa annua, Solanum nigrum* and *Stellaria media*. R biotypes of *Phalaris paradoxa* have been found to be photosynthetically superior to their S counterparts [16]. Jansen *et al.* [17] observed that R *Chenopodium album* chloroplasts had lower electron transport rates between water and plastoquinone compared to S; yet no differences were found in the rate of quantum yield of whole-chain electron transport, or in A, between R and S. These inconsistent responses by R and S biotypes have led several to conclude that the change conferring R is not necessarily directly linked to inferior photosynthetic function [6, 16, 17].

An assessment of these and other studies reveals several possible reasons why different responses may have been observed. They include pleiotropic reorganization of the R chloroplast and the dynamic interrelationship between components of photosynthesis; the role of environment in altering responses; genetic factors, such as differences between biotypes and genome interactions within a biotype; and the possibility of an unnamed factor controlling photosynthesis [8]. Comparison of different photosynthetic responses by R is further complicated by the many different environmental and biological conditions under which they were conducted. These include changes in plant species, plant age, plant uniformity, plant tissue, temperature, PPFD, the degree of environmental control (field, glass-house or controlled environment chambers) and the diurnal light period length and variation of conditions. Another mitigating factor in comparing R and S photosynthetic responses has been the use of model systems in which other genes, besides the mutation to *psb*A, have differed (*e.g.* [3, 18]). Inferences from these studies have been confounded because they relied on a non-isogenic model system. McCloskey and Holt [9] have suggested nuclear genome differences may compensate for differences in productivity between non-isogenic R and S selections, and that detrimental effects may be attenuated by interactions of plastid and nuclear genomes [19]. Many of these limitations may have been overcome in studies with the nearly isonuclear biotypes of *Brassica napus* [7, 19–24].

Pleiotropic reorganization of the R chloroplast

The genetic change in R plants leads to a profound reorganization of functional units in the chloroplast. This adaptive reorganization of photosynthetic components in the chloroplast may be a compensatory mechanism to maintain a functional interaction of the PS II complex lipids and proteins [25]. This pleiotropic cascade includes both structural [26, 27] and functional changes [5]. Several changes in thylakoid lipid chemical composition have been observed. R phospholipids had higher linolenic acid concentrations and lower levels of oleic and linoleic fatty acids [28]. R plants overall were richer in unsaturated fatty acids, had higher proportions (and quantitatively greater amounts of) monogalactosyl diglyceride and phosphatidyl choline, and had lower proportions of di-

galactosyl diglyceride and phosphatidyl choline, than in S [27]. As a consequence of a higher proportion of appressed thylakoids, R plants had a greater proportion of Δ3-*trans*-hexadecenoic acid in phosphatidylglycerol [28]. Although the leaf anatomy of R and S is similar, many ultrastructural characters are different. R has decreased plastid starch content and increased grana stacking (and the associated characters of lower Chl *a*/*b* ratio, increased Chl *a*/*b* light-harvesting complex, and lower P700 Chl *a* and chloroplast coupling factor amounts) [24]. Many of these changes in R are similar to those found in shade-adapted leaves [29]. Seeds from the susceptible biotype apparently germinate earlier and more quickly than those of R [30]. The dynamic nature of the chloroplast to reach a markedly different, new, structural and functional equilibrium in response to the mutation of a key plastidic gene has been observed previously [31–33]. Mattoo [25] has suggested that the rapid anabolism-catabolism rate of the D-1 protein could serve as a signal resulting in the reorganization of membranes around the PS II complex. This dynamic reorganization has consequences for evaluating and understanding regulatory effects of electron transport in carbon assimilation.

Pleiotropy in R: Time, environment and regulation

The equivocal nature of our understanding of how photosynthesis differs between R and S, and how it is regulated, has led us to focus experimental efforts on chronobiological, environmental and regulatory understandings of R under more dynamic, but closely controlled, growth conditions.

Many plant species exhibit an endogenous rhythm of carbon assimilation and stomatal function once entrained in a photoperiod. This rhythm is regulated to some extent independently of the plant's direct response to PPFD [34]. Previous work in our laboratory indicated a consistant, differential, pattern of Chl *a* fluorescence (F_t) [23], carbon assimilation, leaf temperature, total conductance to water vapor (g), and leaf intercellular CO_2 partial pressure (C_i) [21, 22] between S and R *Brassica napus* L. over the course of a diurnal light period, *i.e.* R is a chronomutant. R plants varied in their relative advantage (or disadvantage) over S in terms of carbon assimilation as they aged [22]. It is hypothesized that these ontogenetic and diurnal patterns of differential photosynthesis may be a consequence of correlative diurnal fluctuations in fatty acid biosynthesis and the dynamic changes in membrane lipids over the course of the light-dark daily cycle [35], changes in leaf membrane lipids with age [27], or microenvironmental temperature influences [7, 20, 36].

This research revealed an apparent discrepancy in the response of R to temperature. Carbon assimilation in R was much lower than that in S at high leaf temperatures (*e.g.* 35 °C) when the leaf temperature was closely controlled [7]. These results are consistent with those of others [37–39]. When leaf temperature was not directly controlled, but air temperature was, R carbon assimilation exceeded that of S at relatively high temperatures (*e.g.* 35 °C air temperature) [20, 21]. In both experimental conditions R leaf conductances were usually greater than in S. At relatively cool temperatures it has been hypothesized that the change in lipid saturation of chloroplast membranes could confer cold tolerance to R plants, resulting in greater carbon assimilation rates in R under those conditions [26, 36, 40]. The greater stomatal aperture, and greater leaf conductances apparently negate this effect [7]. As a consequence, at all important physiological temperatures (10–35 °C) R leaves are cooler than S leaves. Stomatal function differentially regulates carbon assimilation ointhese two biotypes.

R adaptation to the environment and regulation of carbon assimilation

Regulation of photosynthesis in R and S are controlled by many different factors in those plants. Limitations in electron transport in R are not the only critical factor in yield losses at the whole plant level. The pleiotropic effects observed in R result in a new equilibrium between functional and structural components. It is this new dynamic pleiotropic reorganization that regulates carbon assimilation in R. Electron transport limitations are only one possible regulatory point in the photosynthetic pathway leading from light-harvesting and the photolysis of water, through ribulose bisphosphate carboxylase/oxygenase, to starch/sucrose biosynthesis, translocation, and utilization. Carbon flux through the leaf is regulated at many points. Electron transport, even in

R, is not the only critical regulatory step. In fact, Dekker and Sharkey [7] have shown that the primary limitation to photosynthesis changes with changes in leaf temperature, and that electron transport limitations in R may be significant only at higher temperatures.

The reorganized R plants interact with the environment in a different way than does S. It is this that causes the functional result observed: under environmental conditions highly favorable to plant growth, S often has an advantage over R. Under certain less favorable conditions to plant growth, stressful conditions, R can be at an advantage over S. It can be envisioned that there were environmental conditions in the absence of *s*-triazine-herbicides in which R had an adaptive advantage over the more numerous S individuals in a population of a species. Under certain conditions R might have exploited a photosynthetic niche under-utilized by S. These conditions may have occurred in less favorable environments and may have been cool (or hot), low light conditions interacting with other biochemical and diurnal plant factors early and late in the photoperiod, as well as more complex physiological conditions late in the plant's development. Under these conditions R survival and continuity could have been ensured at a higher frequency of occurrence than that due to the mutation rate of the *psb*A plastid gene alone, independent of the existence of a postulated plastome mutator [41, 42].

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