Comparing the Photoprotective Importance of Nonphotochemical Quenching and Chloroplast Movement across Plant Species

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A thesis submitted in partial fulfillment of a B.A. degree with honors in Biological Sciences.

May 2012

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While light is the driving force of photosynthesis, excess light can severely damage plants. As sessile organisms exposed to often drastically fluctuating light intensities, plants have evolved several mechanisms for maintaining the delicate balance between maximizing photosynthesis and minimizing photooxidative damage. The xanthophyll cycle allows plants to quickly transition from a state of high photochemical efficiency to one of cautious photoprotection upon changes in light conditions. The associated photoprotective state, known as nonphotochemical quenching, prevents photodamage by innocuously dissipating excess absorbed light energy as heat.

We compared the capacity of *Eichhornia crassipes*, *Hosta ‘Krossa Regal’*, *Taraxacum officinale*, and *Arabidopsis thaliana* wild type, as well as the nonphotochemical quenching and chloroplast movement mutants, *A. thaliana npq1* and *chup1*, to perform nonphotochemical quenching under high light conditions using chlorophyll *a* fluorescence. We also quantified the photoprotective importance of nonphotochemical quenching in each plant by comparing the abilities of leaves treated with dithiothreitol (DTT), an inhibitor of the key xanthophyll cycle enzyme, violaxanthin de-epoxidase, and untreated leaves to recover from high light-induced photoinhibition by measuring chlorophyll *a* fluorescence. Overall, we found that species varied in both the capacity to which they could perform nonphotochemical quenching and the degree to which they could recover from high light exposure, but interestingly, those plants that had greater nonphotochemical quenching abilities did not necessarily demonstrate enhanced resilience to light stress. We also assessed correlations between the abilities of plants to perform nonphotochemical quenching and chloroplast movement, another prominent photoprotective strategy, but did not find that the capacities of plants for these two mechanisms were strongly associated.
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my wonderful advisor, Professor Martina Königer, for her continual support and guidance throughout this project, as well as throughout my time at Wellesley. Her brilliance and dedication have truly inspired me to do my best work both inside and outside of the lab. It has been an honor to be one of her students and to be mentored by such an incredible scientist and person.

I would also like to thank the members of my thesis committee: Professors Simone Helluy, Kaye Peterman, and Ruby Lam. No one has had more confidence in me than Professor Helluy. I cannot thank her enough for her endless encouragement and efforts to keep me balanced. I thank her for getting me out of the Science Center to accompany her on countless walks around Lake Waban, much needed breaks that will be among my greatest memories of Wellesley. I also thank the brilliant Professor Peterman, to whom I owe much of my understanding of plant biology. Her thoughtful questions have helped me to think more deeply about my project. I am also grateful to Lam laoshi for serving on my committee and being a wonderful and patient teacher.

Lastly, I thank my family and friends for their support and encouragement. I am especially grateful to my lab-mates, particularly Caroline Kwon for training me in lab techniques used in this project and Katrina Soriano and Jennifer Yang for helping me collect data.
INTRODUCTION

The relationship between plants and light is highly romanticized; it evokes an idyllic image of vigorous, carefree plants thriving on a bright sunny day, when in reality full sunlight is a potential hazard for most species. While light is the vital element that drives the essential reactions of photosynthesis, ultimately supporting most of life on earth, exposure to too much light can cause severe damage to plants.

The life-supporting process of photosynthesis is an inherently hazardous endeavor for plants and other photosynthetic organisms to undertake, as is any process that involves the generation of highly reactive molecules. When light is absorbed by chlorophyll pigments in the light harvesting complexes, chlorophyll molecules become excited and enter their singlet excited state (\(^1\text{Chl}\)). Singlet excited chlorophyll is usually short-lived, but the transfer of excitation energy amongst surrounding chlorophylls can result in the generation of comparatively stable triplet excited chlorophylls (\(^3\text{Chl}\)) which easily react with oxygen molecules (O\(_2\)), yielding singlet oxygen (\(^1\text{O}_2\)). If singlet oxygen becomes reduced to a superoxide anion (O\(_2^-\)), it can form reactive oxygen species that can cause damage to the photosynthetic apparatus (Niyogi 1999).

While the threat of the formation of destructive reactive oxygen species is ever-present during the process of photosynthesis, the potential for the generation of enough oxidizing molecules to cause substantial damage occurs under conditions of excess light, when light absorption exceeds the amount that can be used safely in photosynthesis. Not only is the magnitude of light energy entering the system greater under excess light conditions than lower light conditions, but when the capacity for photosynthesis becomes overwhelmed, less \(^1\text{Chl}\) can be quenched by photochemistry, the use of energy for photosynthesis at photosystem II, providing a greater
potential for the creation of reactive oxygen species. Thus, excess light makes the potentially hazardous process of photosynthesis even more dangerous.

Despite the risks of this light-induced oxidative damage, known as photooxidation, photosynthetic organisms still exist and thrive in a wide range of light environments. In order to survive as sessile organisms exposed to often drastically fluctuating light intensities, plants must diligently maintain a delicate balance between maximizing photosynthesis and minimizing the light-induced depressions in photosynthetic capacity and resulting damage to the photosynthetic apparatus, collectively known as photoinhibition. Consequently, plants have evolved a variety of photoprotective mechanisms to optimize light absorption for photosynthesis. These mechanisms include both strategies for preventing the absorption of excess light as well as strategies for managing excess absorbed energy when the capacity for photosynthesis becomes saturated (Niyogi 1999).

Avoiding Excess Light Absorption

One way that plants prevent photodamage is by physically avoiding the absorption of excess light. This can be achieved on a large scale, by altering the angle of the plant’s leaves to shelter them from incident light, or on a cellular level through the protective repositioning of organelles. The intracellular rearrangement of chloroplasts, the organelles in which photosynthesis occurs, is a widely recognized photoprotective strategy known as chloroplast movement (Wada et al. 2003), one of the primary photoprotective processes discussed in this paper.

Plants are known to optimize light absorption for photosynthesis through the strategic positioning of their chloroplasts (Wada et al. 2003). Under conditions where light is limited,
plants are observed to spread their chloroplasts across the surface of the cell perpendicular to the incident light. This distribution, known as the face position or accumulation response, maximizes the surface area of chloroplasts exposed to light, consequently maximizing light absorption for photosynthesis. Under conditions where light is no longer limiting, however, a quite different distribution of chloroplasts is typically observed. In order to avoid the absorption of excess light, chloroplasts migrate towards the anticlinal cell walls, decreasing the amount of photosynthetic surface area exposed to light. Known as the profile position or avoidance response, this distribution minimizes light absorption by allowing chloroplasts to shade one another, protecting the cell from the perils of excess light absorption. While the exact mechanisms responsible for chloroplast movement are still being defined, several factors are known to be involved, including: light-sensing phototropins, actin filaments, and the protein CHUP (Wada et al. 2003).

Phototropins Sense Light Conditions

Two photoreceptors, phototropins 1 and 2, which are known to control phototropism, have also been implicated in mediating chloroplast photorelocation movements (Sakai et al. 2001). These two photoreceptors are protein kinases that are able to sense blue light conditions via two light, oxygen, and voltage (LOV) domains that are activated following the absorption of blue light (Briggs and Christie 2002). Experiments with Arabidopsis thaliana phototropin mutants, phot1 (phototropin 1 mutant), phot 2 (phototropin 2 mutant), and phot1/phot2 (phototropin 1 and 2 double mutant) have determined that the functions of these structurally similar photoreceptors are partially redundant, with both photoreceptors controlling the chloroplast accumulation response, but only phototropin 2 mediating the avoidance response.
(Sakai et al. 2001). While the mechanism by which activated phototropins trigger chloroplast movement has yet to be fully characterized, signaling via calcium ions (Ca\(^{2+}\)) is thought to be involved (Stoelzle et al. 2003; Tłałka and Gabryś 1993).

The Role of Actin in Chloroplast Positioning

While the precise mechanism by which chloroplast movement occurs has yet to be fully defined, experimental evidence supports the involvement of actin filaments (Kadota et al. 2009; Kandasamy and Meagher 1999; Kobayashi et al. 2009; Tłałka and Gabryś 1993). It has been proposed that chloroplasts move along actin cables, possibly with the help of motor molecules such as myosins, as immunofluorescence imaging has shown that some chloroplasts align with actin filaments in A. thaliana (Kandasamy and Meagher 1999) and treatment of leaves with myosin inhibitors has been known to disrupt chloroplast movement, particularly inhibiting the accumulation response (Paves and Truve 2007). Short actin filaments have been observed to appear on the leading edge of relocating chloroplasts, further implicating the role of actin and suggesting that the formation of small pieces of actin closely associated with chloroplasts may provide a mechanism for their rearrangement (Kadota et al. 2009). The formation of these intimately associated short actin filaments has been found to be dependent on phototropins 1 and 2, further suggesting that they are involved in light-directed chloroplast movement (Kadota et al. 2009). Visualizations of actin and chloroplasts have also revealed that some chloroplasts, especially those in mesophyll cells, appear to be wrapped in ‘baskets’ of actin, suggesting that actin may serve an anchoring role in chloroplast positioning (Kandasamy and Meagher 1999). Furthermore, treatment of plants with actin depolymerizing drugs, such as cytochalasin B and Latrunculin B, have been observed to disrupt actin-chloroplast associations and inhibit
chloroplast movement, suggesting that actin is indeed involved in the intracellular localization of chloroplasts (Kandasamy and Meagher 1999; Kobayashi et al. 2009). While the involvement of actin in chloroplast movement has been well established, the exact mechanism by which they move is still unclear.

Further evidence for the involvement of actin in chloroplast movement can be deduced from experiments with A. thaliana chup1 (chloroplast unusual positioning) mutants, which lack an actin-binding protein found in the outer chloroplast envelope that potentially connects chloroplasts to actin cables and anchors them to the plasma membrane (Oikawa et al. 2003; Oikawa et al. 2008; Schmidt von Braun and Schleiff 2008). The CHUP1 protein may also be involved in actin polymerization, as it has been observed to bind to the actin modifying protein, profilin (Schmidt von Braun and Schleiff 2008). A. thaliana chup1 mutants fail to exhibit chloroplast movement in response to changing light intensities and are characterized by an atypical intracellular distribution of chloroplasts in which chloroplasts are localized in clusters near the bottom of cells (Oikawa et al. 2003). These mutants also fail to form the short chloroplast associated actin filaments that are thought to be involved in chloroplast rearrangement (Kadota et al. 2009). The observations of elimination of chloroplast movement and abnormal chloroplast positioning in an actin-binding protein mutant further confirm the importance of actin in this photoprotective strategy.

Microtubules may also have a role in chloroplast positioning, though the evidence of microtubule involvement is debated. While the chemical disruption of microtubules did not appear to alter the positioning of chloroplasts in A. thaliana (Kadota et al. 2009; Kandasamy and Meagher 1999), treating the moss Physcomitrella patens, with Cremart, a microtubule depolymerizing drug, disrupted chloroplast movement, particularly in the longitudinal direction.
(Sato et al. 2001). Thus, while it is clear that actin is involved in chloroplast positioning, microtubules may also have a role in mosses, but not in higher plants.

The Importance of Chloroplast Movement

Experiments with A. thaliana mutants with impaired chloroplast movement have confirmed the photoprotective importance of the chloroplast avoidance response under high light conditions (Kasahara et al. 2002). Exposure of phot2 and chup1 mutants, which are deficient in performing the avoidance response, to extended high light conditions resulted in visibly photodamaged leaves. The leaves of phot2 and chup1 plants suffered bleaching and necrosis after only 10 hours of high light stress, whereas the leaves of wild type plants appeared undamaged, even after more than 30 hours of excess light exposure. The phot1 mutant, in which chloroplast avoidance movements are uninhibited, did not exhibit symptoms of photodamage as the other chloroplast movement mutants did, suggesting that the avoidance response in particular is important in photoprotection (Kasahara et al. 2002).

Ecological Patterns in the Capacity for Chloroplast Movement

Chloroplast movement is common to a wide range of plant species including ferns, aquatic plants, mosses, and C3, C4, and CAM plants, as well as other photosynthetic organisms such as algae (Kobayashi et al. 2009; Kondo et al. 2004; Park et al. 1996; Sharon and Beer 2008; Wada et al. 2003), but species vary greatly in their capacities to reposition their chloroplasts. Different species show wide variation in both the speed and extent of their chloroplast movements, as well as the types of avoidance positions their chloroplasts can assume.
Differences in light environments may drive some of the variation in chloroplast movement abilities observed between species. In comparing the shade plant *Tradescantia albiflora* to the sun-loving plant pea (*Pisum sativum*), Park et al. (1996) found that *T. albiflora* exhibited superior light stress tolerance as well as a greater capacity for chloroplast rearrangement. This suggests that chloroplast movement helps to protect plants from photoinhibition and may be an important strategy employed by shade plants to protect their leaves from high light intensities that easily overwhelm their limited capacities for photosynthesis. Chloroplast movement may also be particularly important for plants that live in environments with variable light intensities. In a study of fern species, it was observed that those ferns that exhibited the greatest environmental flexibility and had the broadest habitat distributions were able to rearrange their chloroplasts with greater speed and to a greater extent than their counterparts with narrower light requirements (Augustynowicz and Gabryś 1999). The results of these studies suggest that the capacity of species to relocate their chloroplasts in response to changes in the intensity of incident light may be correlated with factors in their natural environment, especially light, and that chloroplast movement may be a mechanism by which species adapt to their environment.

Not only do species differ in the speed and degree to which they can move their chloroplasts, but some species also differ in their intracellular chloroplast arrangements, deviating from the standard profile position avoidance response. An alternative avoidance response in which chloroplasts congregate into one or more large clumps within a cell upon exposure to high light have been observed in several species of succulent CAM photosynthesizing plants under water-stress (Kondo et al. 2004) as well as in the seagrass, *Halophila stipulacea* (Sharon and Beer 2008). The clumping arrangement of chloroplasts can
reduce light absorption under excess light conditions by reducing the surface area of chloroplasts exposed to the light as well as by allowing chloroplasts to shade one another. Thus, while the standard anticlinal avoidance response is typical among most species studied thus far, alternative avoidance positions have been observed.

A recent comparison of chloroplast photorelocation movements across a broad range of species by Königer and Bollinger (2012) confirmed the observation that chloroplast movement is not a uniform process. While there was great variation in both the speed and amplitude with which species were able to mobilize their chloroplasts, those species that exhibited greater capacities to perform chloroplast movement did not necessarily display an enhanced ability to recover from high light stress (Königer and Bollinger 2012). Kwon (2011) expanded on this study by quantifying the photoprotective importance of chloroplast movement in the same species. Treating leaves with the actin depolymerizing agent cytochalasin B to selectively inhibit chloroplast rearrangement, high light stress treatments were performed and revealed that chloroplast movement was indeed a critical process for many species. Obliterating the ability of many plants to move their chloroplasts dramatically decreased their abilities to recover from high light exposure. However, some species were still able to make a substantial recovery from high light stress even in the absence of chloroplast movement, which suggests that other mechanisms are involved in protecting plants from excess light and may have greater photoprotective importance in some species (Kwon 2011).

**Managing Excess Light Absorption**

Despite the efficiency of mechanisms plants use to prevent excess light absorption, these strategies are not infallible and the absorption of light that exceeds the capacity for
photochemistry is a common occurrence under high light conditions. Consequently, plants have evolved additional photoprotective strategies for managing excess light post-absorption, the most prominent one being nonphotochemical quenching, a process by which excess absorbed energy can be innocuously dissipated as heat (Demmig-Adams 1990).

The Xanthophyll Cycle

Nonphotochemical quenching is facilitated, to a large extent, via the xanthophyll cycle. This universal cycle, also known as the violaxanthin cycle, involves the reversible interconversion of three main carotenoid pigments: violaxanthin, antheraxanthin, and zeaxanthin, all of which are localized in the light harvesting antenna (Demmig-Adams 1990). The pigment violaxanthin is a di-epoxide that is capable of harvesting light energy for photochemistry (Owens et al. 1987). This pigment dominates in the xanthophyll pigment pool during periods of limited light, conditions that favor photosynthesis rather than photoprotection and in which photochemical efficiency is high (Demmig-Adams 1990; Demmig-Adams and Adams 1996; Li et al. 2009). Under conditions of excess light, the rapid splitting of water molecules at the reaction center of photosystem II and the reduction of plastoquinone to plastoquinol, coupled with the relative lag in the return of protons to the thylakoid space by ATP synthase, creates a proton gradient (ΔpH) across the thylakoid membrane. The increasingly acidic conditions of the thylakoid space triggers the activation of the enzyme violaxanthin de-epoxidase, which catalyzes the conversion of violaxanthin to the mono-epoxide intermediate, antheraxanthin, as well as the de-epoxidation of antheraxanthin to the epoxide-free pigment, zeaxanthin, which rapidly accumulates to compose most of the xanthophyll pigment pool under high light conditions.
The bulk of nonphotochemical quenching is mediated by zeaxanthin, which has the ability to dissipate absorbed energy as heat (Demmig-Adams 1990; Li et al. 2000). While zeaxanthin is inefficient in channeling energy towards photochemistry unlike its di-epoxide counterpart, violaxanthin, the pigment’s ability to essentially ‘waste’ energy is valuable when the capacity for photosynthesis becomes overwhelmed. Safely removing excess energy through thermal dissipation is an innocuous alternative to creating reactive oxygen species and consequently threatening the integrity of the cell. It has been hypothesized that the structure of zeaxanthin, which contains more conjugated double bonds than the other two xanthophyll cycle pigments, allows it to dissipate heat and efficiently de-excite singlet oxygen (Krinsky 1979). As a structural intermediate between violaxanthin and zeaxanthin, antheraxanthin may also be responsible for some of the nonphotochemical quenching that occurs under excess light (Demmig-Adams and Adams 1996; Kato et al. 2003).

Upon the return of light limited conditions, the xanthophyll cycle typically undergoes a rapid reversal characterized by the relaxation of nonphotochemical quenching. The zeaxanthin accumulated under high light is reconverted to antheraxanthin, and antheraxanthin to violaxanthin, via the enzyme zeaxanthin epoxidase (Eskling et al. 1997). This fast and efficient conversion often occurs in a matter of minutes, allowing plants to quickly return from a state of cautious photoprotection to one of high photochemical efficiency (Nilkens et al. 2010).
The Different Types of Nonphotochemical Quenching

Nonphotochemical quenching can be divided into several distinct types which are most easily differentiated based on the kinetics of the induction and relaxation of thermal quenching: a fast, intermediate speed, and slow process (Nilkens et al. 2010; Quick and Stitt 1989). These different components, which are known to be driven by different underlying mechanisms, are officially known as qE, qZ, and qI, respectively (Table 1).

### TABLE 1. Summary of the different types of nonphotochemical quenching.

<table>
<thead>
<tr>
<th>Type</th>
<th>Alternative names</th>
<th>Time frame of induction and relaxation</th>
<th>Characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>qE</td>
<td>ΔpH-dependent NPQ, rapidly reversible NPQ, feedback de-excitation, flexible dissipation, high-energy state quenching, energy-dependent quenching</td>
<td>Seconds to minutes</td>
<td>ΔpH-dependent, requires PsbS, has both zeaxanthin-dependent and zeaxanthin-independent components</td>
<td>Nilkens et al., 2010</td>
</tr>
<tr>
<td>qZ</td>
<td>N/A</td>
<td>Tens of minutes</td>
<td>Involves de novo synthesis of zeaxanthin, ΔpH-dependent</td>
<td>Nilkens et al., 2010</td>
</tr>
<tr>
<td>qI</td>
<td>Photoinhibitory quenching, sustained thermal dissipation, slow relaxation</td>
<td>Hours or longer</td>
<td>Due to reversible photoinhibitory damage to the reaction center of photosystem II</td>
<td>Krause, 1988</td>
</tr>
</tbody>
</table>
The most substantial type of nonphotochemical quenching is qE, which typically accounts for 70-80% of total nonphotochemical quenching (Li et al. 2000; Nilkens et al. 2010). qE is characterized by its rapid induction and reversibility and is mediated by changes in the epoxidation state of the xanthophyll pigments. After exposure to high light, qE is typically induced within a matter of seconds as zeaxanthin is rapidly produced via xanthophyll cycle activity (Nilkens et al. 2010). Nilkens et al. (2010) estimated that qE is fully induced 10-200 seconds after exposure to high light. Likewise, the qE component of nonphotochemical quenching is also rapidly reversible, relaxing within approximately 30-60 seconds after exposure to dark conditions, and strongly associated with the collapse of ΔpH (Nilkens et al. 2010).

qE is dependent on a trans-thylakoid pH gradient, as ΔpH induces xanthophyll conversions, as well as a protein called PsbS (Li et al. 2000). PsbS is a protein found in the light harvesting complex of photosystem II that has been correlated with capacity for qE in a dosage-dependent manner (Li et al. 2002). PsbS has also been shown to influence the induction and relaxation kinetics of qE, as illustrated in studies of mutants with variable expression of PsbS (Nilkens et al. 2010; Zia et al. 2011). While the mechanistic relationship between PsbS and nonphotochemical quenching is still unclear, it has been suggested that PsbS is the site at which qE occurs as PsbS has been observed to bind to xanthophyll cycle pigments (Li et al. 2000).

Another hypothesis is that PsbS may catalyze the rearrangement of photosystem II supercomplexes into formations conducive to nonphotochemical quenching, as the PsbS content of plants has been associated with differently organized grana membranes (Kereiche et al. 2009).

The intermediate speed component, qZ is characterized by the de novo synthesis of zeaxanthin (Nilkens et al. 2010). Nilkens et al. (2010) estimated that qZ is induced within 10-30 minutes of exposure to excess light and is responsible for approximately 20-25% of total
nonphotochemical quenching in *A. thaliana*. Like qE, the qZ component of nonphotochemical quenching is also dependent on both ΔpH and zeaxanthin, but does not require PsbS. qZ relaxation occurs fully within 10-60 minutes of the restoration of light-limited conditions as zeaxanthin is epoxidized to non-quenching xanthophyll carotenoids (Nilkens et al. 2010).

qI, or photoinhibitory quenching, is the slowest type of nonphotochemical quenching with an induction time of hours or longer. qI is a side effect of photoinhibition, a decrease in photochemical efficiency due to damage to the reaction centers of photosystem II as a result of prolonged excess light exposure (Krause 1988). Although the inactivated reaction centers can no longer effectively harvest light for photosynthesis, they can serve a photoprotective role by continuing to absorb light energy and safely releasing it through thermal dissipation. The relaxation of qI is gradual compared to the other components of nonphotochemical quenching, as it requires the repair of damaged reaction center proteins (Krause 1988).

While most thermal energy dissipation is mediated by zeaxanthin, not all nonphotochemical quenching is zeaxanthin-dependent (Adams et al. 1990; Demmig-Adams et al. 1990; Johnson et al. 2009; Li et al. 2000). Evidence for the existence of a zeaxanthin-independent nonphotochemical quenching mechanism has been revealed in studies showing that leaves treated with dithiothreitol (DTT), a chemical inhibitor of violaxanthin de-epoxidase, are still able to perform thermal energy dissipation, albeit to a much lesser extent (Adams et al. 1990; Demmig-Adams et al. 1990). It has been suggested that in addition to zeaxanthin, a structurally similar carotenoid pigment, lutein, may also be responsible for some thermal quenching activity (Johnson et al. 2009; Li et al. 2009; Pogson et al. 1998). Li et al. (2009) found that enhancing lutein production in zeaxanthin-deficient *A. thaliana* mutants partially restored the plants’ capacities for qE, suggesting that the lutein may play a similar role to zeaxanthin in
nonphotochemical quenching. Lutein deficient mutants have been observed to have decreased capacities for thermal dissipation and appear to be particularly impaired in the induction phase of qE, suggesting that lutein may play a role in the rapid induction of nonphotochemical quenching (Pogson et al. 1998).

The Importance of Nonphotochemical Quenching

The photoprotective importance of nonphotochemical quenching has been elegantly illustrated through studies of mutant plants with attenuated capacities for thermal dissipation. These studies demonstrate the importance of nonphotochemical quenching for growth and survival in environments with variable light intensity and show that the ability to perform nonphotochemical quenching has adaptive significance.

Plants that lack the ability to accumulate zeaxanthin under high light conditions suffer damage of greater severity from excess light than those with normally functioning xanthophyll cycles. Studies with Arabidopsis thaliana nonphotochemicalquenching1 (npq1) mutants, which are unable to produce violaxanthin de-epoxidase and therefore exhibit impaired xanthophyll cycle activity (Niyogi et al. 1998), have confirmed the photoprotective importance of nonphotochemical quenching. A. thaliana npq1 plants grown under high light conditions have been observed to experience greater photoinhibition and suffer more photooxidative damage and lipid peroxidation than their wild type counterparts (Havaux et al. 2000; Havaux and Niyogi 1999; Niyogi et al. 1998). Similarly, A. thaliana npq4 mutants, which are deficient in PsbS proteins, have shown greater susceptibility to photoinhibition in full sunlight (Külheim et al. 2002). Mutants with atypical compositions of xanthophyll pigments, and consequently reduced capacities for nonphotochemical quenching, have also been observed to experience delayed
development, and even increased mortality, compared to plants with unaltered pigment compositions, further highlighting the importance of this photoprotection mechanism (Pogson et al. 1998).

Ultimately, the ability to perform nonphotochemical quenching has demonstrated adaptive significance. Field experiments with A. thaliana npq1 and npq4 mutants have shown that the ability to thermally dissipate excess energy confers a fitness advantage as these mutants deficient in nonphotochemical quenching annually produced 30-50% fewer seeds and 25% fewer fruits than their wild type counterparts (Frenkel et al. 2009; Külheim et al. 2002). Laboratory experiments have shown that the fitness advantage of unimpaired nonphotochemical quenching become particularly pronounced when plants are grown under conditions with variable light intensity (Külheim et al. 2002). It has been suggested that these differences in reproductive output may be at least partially attributed to photooxidative stress, which may cause plants to allocate less resources towards seed production (Frenkel et al. 2009). This redirection of metabolism may be mediated by jasmonic acid, similar to the plant wounding stress response that triggers the preferential allocation of resources towards defense, as an upregulation of genes in the jasmonic acid biosynthesis pathway was observed in light-stressed npq4 mutants (Frenkel et al. 2009). Regardless of the mechanism, it is clear that the ability to perform nonphotochemical quenching is highly advantageous for plants, especially under variable light conditions.

Ecological Patterns in the Capacity for Nonphotochemical Quenching

While nonphotochemical quenching seems to be a universal photoprotective mechanism amongst plants, the ability to perform nonphotochemical quenching has been observed to vary
greatly amongst different species and individual plants growing in different light environments (Demmig-Adams 1998; Demmig-Adams and Adams 1996; Königer et al. 1995). In general, plants grown under higher light intensities tend to have greater capacities for thermal energy dissipation than their shade-grown counterparts.

At the organismal level, leaves grown in direct sunlight have been observed to be able to induce nonphotochemical quenching faster and to a greater degree than shade-grown leaves of the same species (Demmig-Adams 1998). This enhanced ability to cope with excess light absorption may be attributed to the greater size of xanthophyll cycle pigment pools found in individual plants grown in high light compared to those grown under light-limited conditions, a pattern that has observed across a range of species including A. thaliana, creeping holly (Mahonia repens), periwinkle (Vinca minor), and Chenopodium album (Demmig-Adams 1998; Golan et al. 2006; Kato et al. 2003). These observations suggest that nonphotochemical quenching is a useful tool for plants to acclimate to different light conditions.

Differences in the capacity of plants to perform nonphotochemical quenching become even more pronounced when comparing different species, especially those with different life history strategies and those from different environments. It has generally been found that long-lived and slow growing species tend to have greater abilities to perform qE compared to short-lived species that grow quickly (Demmig-Adams and Adams 2006). The capacity of species to thermally dissipate excess energy also appears to be related to environmental factors. Königer et al. (1995) found dramatic differences in the xanthophyll cycle pigment pool sizes of plants in different light environments of the tropical rainforest: the canopy, gaps, and the understory. Canopy trees, which had the highest photosynthetic capacities, had xanthophyll pigment pools almost 40% larger than those of species that colonized gaps or the understory, with understory
plants having the smallest overall pools (Königer et al. 1995). Similarly, Demmig-Adams (1998)
found that species growing in environments with low light intensities had smaller xanthophyll
pools and slower nonphotochemical quenching kinetics than those that grew in sunny areas.
Perhaps the most extreme variations in nonphotochemical quenching occur in slow-growing
evergreen trees exposed to severe and prolonged environmental stress. Overwintering conifers,
for example, have been known to almost completely down regulate photosynthesis and adopt a
state of sustained thermal dissipation during which high levels of zeaxanthin are maintained,
even in the absence of light (Demmig-Adams and Adams 2006). These differences in
photoprotective behavior suggest that nonphotochemical quenching is an important component
of environmental adaptation.

*Other Strategies for Managing Excess Light Absorption*

While nonphotochemical quenching is an efficient mechanism for preventing
photoxiation when the amount of light absorbed by a plant exceeds the amount that can be used
for photochemistry, the capacity for nonphotochemical quenching can also become overwhelmed
during high light exposure. In experiments with *Chenopodium album*, Kato et al. (2003) found
that the amount of excess absorbed light energy that the plants were unable to release via
nonphotochemical quenching was correlated with the rate of photoinhibition. However, many
plants have additional lines of defense against photodamage beyond thermal dissipation and are
able to mitigate the consequences of excess light absorption.

Many plants are able to prevent damage by scavenging reactive oxygen species with
antioxidant systems. Xanthophyll carotenoids, including zeaxanthin, have been implicated in
preventing oxidative damage, independent of their roles in nonphotochemical quenching
Johnson et al. (2007) found that *A. thaliana sChyB* mutants, which produce elevated levels of zeaxanthin despite exhibiting wild type levels of nonphotochemical quenching, displayed enhanced tolerance to light stress, suggesting that zeaxanthin may protect plants from photodamage by an additional mechanism. *sChyB* mutants were observed to exhibit lower levels of lipid peroxidation compared to wild type plants, which has led to formation of the hypothesis that zeaxanthin may bind to lipids in the PSII antennae, protecting them from damaging reactive oxygen species (Johnson et al. 2007). Mutants deficient in nonphotochemical quenching have been observed to increase production of antioxidants, partially compensating for deficiencies in photoprotective abilities, demonstrating the importance and efficacy of antioxidant systems (Golan et al. 2006).

Plants also have the ability to reverse the consequences of photodamage by repairing damaged proteins in photosystem II, particularly the D1 protein localized at the reaction center that is highly susceptible to damage (Greer et al. 1986; Niyogi 1999). Studies have shown that the treatment of plants with the protein synthesis inhibitor, chloramphenicol (CAP), decreases the ability of plants to recover from high light exposure (Demmig-Adams and Adams 1993; Greer et al. 1986). It has also been suggested that plants with lower capacities for other photoprotective mechanisms may compensate by having greater abilities to repair damaged proteins. For example, the sun plant *P. sativum*, which was observed to have a lesser capacity to perform chloroplast movement than its shade tolerant counterpart *T. albifolia*, was observed to utilize D1 synthesis to a greater degree (Park et al. 1996).
Experimental goals

It is clear that exposure to high light intensities poses a substantial threat to plants and that multiple mechanisms are involved in protecting the photosynthetic apparatus from the perils of excess light absorption. Kwon (2011) found that chloroplast movement was an important component of allowing plants to recover from photoinhibition following high light stress, for many species, but that chloroplast movement could not exclusively account for the entire observed recovery from light stress. Some species were able to recover from photoinhibition almost completely, even when chloroplast movement was inhibited (Kwon 2011). Therefore, in this study we sought to investigate the role that nonphotochemical quenching plays in allowing plants to recover their photosynthetic efficiency following high light exposure with the ultimate goal of comparing the relative photoprotective importance of nonphotochemical quenching and chloroplast movement. It is possible that those plants that are able to move their chloroplasts with great facility do not need to utilize nonphotochemical quenching to as large an extent as their counterparts with lower capacities for chloroplast movement because they are able to effectively prevent photodamage through avoidance mechanisms. On the other hand, less facile chloroplast movers may adopt a strategy in which enhanced abilities to manage excess energy post-absorption may compensate for deficiencies in avoidance mechanisms.

We compared the abilities of some of the same species and mutants as Kwon (2011), A. thaliana wild type, npq1, and chup1, Eichhornia crassipes (water hyacinth), Hosta ‘Krossa Regal’, and Taraxacum officinale (common dandelion), to perform nonphotochemical quenching under high light conditions using chlorophyll a fluorescence. The measurement of chlorophyll a fluorescence is a technique that allows one to quantify the amount of absorbed photons that are quenched by photochemistry versus dissipated thermally, based on the fact that some of the
absorbed energy will be released as fluorescence, and is commonly used to quantify both nonphotochemical quenching as well as the yield of photosynthesis (Baker 2008). In order to specifically elucidate the photoprotective importance of nonphotochemical quenching, we also quantified the abilities of the preceding species to recover from high light-induced photoinhibition when treated with dithiothreitol (DTT), an inhibitor of qE (Adams et al. 1990), by measuring the yield of photosynthesis after high light exposure using chlorophyll $a$ fluorescence. By comparing the abilities of DTT-treated leaves to recover to those of Kwon’s (2011) cytochalasin B-treated leaves, we can quantitatively compare the relative importance of nonphotochemical quenching and chloroplast movement in protecting a wide range of plant species from excess light.
MATERIALS & METHODS

Plant Materials

Plants were obtained from a variety of sources (Table 2). Wild type *Arabidopsis thaliana* and *chup1* T-DNA insertional mutant seeds (stock number SALK_10504) (Alonso et al. 2003) were purchased from the Arabidopsis Biological Resource Center (ABRC, www.abrc.osu.edu). *A. thaliana npq1* mutant seeds were purchased from The Arabidopsis Information Resource (TAIR, www.arabidopsis.org) (stock number CS3771). *Eichhornia crassipes* (water hyacinth) plants were obtained from the Margaret C. Ferguson Greenhouses at Wellesley College (Wellesley, MA, USA). *Hosta ‘Krossa Regal’* plants were purchased from Windy Lo Nursery in Natick, MA, USA. Wild *Taraxacum officinale* (common dandelion) plants were collected from outside the Science Center at Wellesley College (Wellesley, MA, USA).
TABLE 2. Species and mutant information.

<table>
<thead>
<tr>
<th>Species/genotype</th>
<th>Family</th>
<th>Characteristics</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arabidopsis thaliana</em> wild type (WT)</td>
<td>Brassicaceae</td>
<td>Eudicot, annual</td>
<td>The Arabidopsis Biological Resource Center</td>
</tr>
<tr>
<td><em>Arabidopsis thaliana</em> nonphotochemicalquenching1 (npq1)</td>
<td>Brassicaceae</td>
<td>Mutant deficient in violaxanthin de-epoxidase</td>
<td>The Arabidopsis Information Resource Center</td>
</tr>
<tr>
<td><em>Arabidopsis thaliana</em> chloroplastunusualpositioning1 (chup1)</td>
<td>Brassicaceae</td>
<td>Mutant with atypical chloroplast positioning and impaired chloroplast movement</td>
<td>The Arabidopsis Biological Resource Center</td>
</tr>
<tr>
<td><em>Eichhornia crassipes</em> (water hyacinth)</td>
<td>Pontederiaceae</td>
<td>Monocot, perennial, hydrophyte, invasive, grows in sun</td>
<td>Margaret C. Ferguson Greenhouses, Wellesley College, Wellesley, MA, USA</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>Liliaceae</td>
<td>Monocot, perennial, herb, grows in shade</td>
<td>Windy Lo Nursery, Natick, MA, USA</td>
</tr>
<tr>
<td><em>Taraxacum officinale</em> (common dandelion)</td>
<td>Asteraceae</td>
<td>Eudicot, perennial, weed, grows in sun</td>
<td>Wellesley College, Wellesley, MA, USA</td>
</tr>
</tbody>
</table>

Growth Conditions

Plants were grown under conditions designed for optimal growth. *A. thaliana* WT, *npq1*, and *chup1*, as well as *Hosta ‘Krossa Regal’* plants were planted in Metro-Mix 360 (Sun Gro Horticulture, Bellevue, WA, USA) and grown under daily cycles of 8 h of light (170 μmol photons m\(^{-2}\)s\(^{-1}\)) and 16 h dark with day temperatures of 25°C and night temperatures of 23°C. Relative humidity was approximately 30%. *E. crassipes* plants were maintained in container of tap water, supplemented with a small amount of Metro-Mix 360 to provide nutrients, and grown
under daily light cycles of 12 h of light (400 μmol photons m$^{-2}$s$^{-1}$) and 12 h dark with day and night temperatures of 22°C and 50% relative humidity. The preceding plants were fertilized weekly with an all-purpose fertilizer (Peters 20-20-20). *T. officinale* plants grew naturally outdoors and were collected during the fall of 2011.

*Treatment of Leaf Discs*

Healthy, fully-expanded leaves were cut into 6 mm diameter circular discs with a cork borer and floated in buffer (100 mM KCl, 1mM CaCl$_2$, 5mM KH$_2$PO$_4$ adjusted to pH 7 with NaOH) on a shaker at 50 M$_{o}$t min$^{-1}$ for 1 h under low light (1.5 μmol photons m$^{-2}$s$^{-1}$) to convert any previously accumulated zeaxanthin back to violaxanthin. After 1 h the buffer was replaced with a 40 mM DTT (DL-dithiothreitol, Aldrich Chemical Company, Allentown, PA, USA) solution (diluted from a 1000 mM aqueous DTT stock solution with buffer) with 0.1% Tween detergent (Sigma Chemical Co., St. Louis, MO, USA) and then floated under low light for an additional 1.5 h. The abilities of DTT concentrations ranging from 1 mM to 40 mM to inhibit nonphotochemical quenching were initially assessed and 40 mM appeared to inhibit nonphotochemical quenching most sufficiently across species (Figure 2; Kwon 2011). After flotation under low light, the DTT solution was forced into the leaf discs via vacuum infiltration. Sufficiently vacuum infiltrated leaf tissue was observed to sink when suspended in the DTT solution. As a control, leaf discs were floated in buffer with 0.1% Tween under the same conditions for 1.5 h, instead of DTT solution, and subsequently vacuum infiltrated. All leaf discs were washed with buffer following vacuum infiltration and maintained in buffer under dark conditions prior to measurements.
Measurement of Nonphotochemical Quenching Kinetics

In order to compare the capacities of different plants to thermally dissipate excess light energy, nonphotochemical quenching kinetics were observed in each species under identical light conditions. Nonphotochemical quenching was measured via chlorophyll $a$ fluorescence with a modulated fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). Treated leaf discs were secured in a leaf clip atop a piece of wet filter paper and dark adapted (0-1 μmol photons m$^{-2}$s$^{-1}$) for 10 min and exposed to a 10 s pulse of far red light, after which the unquenched maximal yield of fluorescence ($F_m$) was measured. The maximal quantum yield (maximum quantum photochemical efficiency of photosystem II), $F_v/F_m$, was also calculated to ensure the leaves were not stressed by the preceding treatment or other environmental factors (Baker 2008; Björkman and Demmig 1987; Butler and Kitajima 1975). Immediately after the initial dark adaptation, leaf discs were exposed to high light (1000 μmol photons m$^{-2}$s$^{-1}$) from an external halogen lamp for 12 min, during which the quenched maximal yield of fluorescence ($F_m'$) was measured minutely. The high light treatment was followed by a 15 minute recovery period during which the leaf discs were returned to dark conditions and exposed to a second 10 s pulse of far red light and minutely $F_m'$ measurements were continued. The temperature of the leaf discs was maintained at approximately 20°C throughout the trial with the aid of a fan to combat increased heat from the external light source during the high light treatment and the filter paper underneath the leaf disc was moistened as needed with water to protect the leaf tissue from desiccation. Measurements were performed on both control and DTT-treated leaf discs in order to measure the capacity of species to perform nonphotochemical quenching and ensure that DTT adequately inhibited nonphotochemical quenching, respectively. The amount of
nonphotochemical quenching was quantified using the Stern-Vollmer relationship (Bilger and Björkman 1990):

\[ NPQ = \frac{Fm - Fm'}{Fm'} \]

The capacity of each species to perform nonphotochemical quenching was determined by averaging the maximum NPQ value (NPQ_{\text{max}}) achieved during the high light treatment of each replicate. The average amount of slowly reversible nonphotochemical quenching performed by each species was determined as the amount of nonphotochemical quenching that did not relax by the end of the 15 minute recovery period. Mean nonphotochemical quenching capacities were compared between species and DTT-treatments using two-way ANOVA tests (JMP; SAS, Cary, NC, USA).

*Measurement of Photochemical Quenching in High Light*

In order to determine the ability of plants to use absorbed light for photosynthesis, the coefficient of photochemical fluorescence quenching (qP), a value ranging from 0 to 1 and estimates the proportion of the maximal efficiency of photosystem II that is achieved, was determined via chlorophyll a fluorescence at the end of the 12 min high light period under the same conditions used to measure nonphotochemical quenching kinetics (described in previous section). qP was determined by measuring the minimal fluorescence yield (Fo) at the end of the initial dark adaptation and instantaneous fluorescence (Ft) and Fm’ after 12 min of high light exposure. qP was calculated using the following equation (Oxborough and Baker 1997):

\[ qP = \frac{Fm' - Ft}{Fm' - Fo} \]
Mean photochemical quenching capacities were compared between species and DTT-treatments using a two-way ANOVA test.

Measurement of Recovery from High Light Stress

In order to quantify the photoprotective importance of nonphotochemical quenching, we performed high light stress treatments on leaf discs treated with DTT, as well as buffer-treated controls, paralleling the structure of experiments performed by Kwon (2011). Treated leaf discs were placed atop a piece of filter paper that was continually moistened with water to prevent desiccation and enclosed in a treatment chamber that was flushed with humidified air to maintain a constant temperature of 20°C (Königer et al. 1998). Leaf discs were initially exposed to low light (10 μmol photons m⁻²s⁻¹) for 30 min, during which the effective quantum yield of photosystem II (yield), a reliable indicator of overall photosynthetic yield under continuous light conditions, was computed every 10 min by measuring Fm’ and Ft via chlorophyll a fluorescence. Leaf discs were then exposed to 90 min of high light (1000 μmol photons m⁻²s⁻¹) and during which the yield was calculated every 30 min. Subsequent to the high light treatment, leaf discs were returned to low light conditions for a 60 min recovery period during which the yield was measured every 10 min. Yield was calculated using the following equation (Baker 2008):

\[ \text{Yield} = \frac{Fm' - Ft}{Fm'} \]

The ability of plants to recover from high light stress was quantified by calculating the percent recovery, determined by dividing the yield at the end of the 60 min recovery period by the yield at the end of the initial 30 min low light acclimation period prior to high light exposure.
Percent recovery values were compared between species and treatments using two-way ANOVA tests.

*Measurement of Chloroplast Movement via Leaf Transmission*

In order to compare our results with the findings of Kwon’s 2011 study of the photoprotective importance of chloroplast movement, it was important to ensure that DTT did not disrupt the ability of plants to relocate their chloroplasts. Therefore, we measured the transmission of light through leaf discs treated with DTT exposed to increasing light intensities and compared the results with those observed in buffer-treated leaves in which chloroplast movement should not be inhibited. The optical properties of a leaf can be used as a measure of chloroplast movement because the amount of light allowed through a leaf changes as chloroplasts rearrange intracellularly: the avoidance response is characterized by a relatively high transmission of light, as light is allowed through the leaf as chloroplasts migrate towards the anticlinal cell walls, whereas transmission is comparatively lower in leaves in which chloroplasts are arranged in the accumulation response, as the wide distribution of chloroplasts in the face position blocks the passage of light through the leaf. We measured the transmission of light through leaves using a microcontroller-based photometric instrument specially designed for monitoring chloroplast movement (Berg et al. 2006). Leaf discs were secured in a leaf clip so that the adaxial leaf surface was exposed to a red and blue light emitting diode (LED), the light intensity of which was manipulated to invoke blue light-dependent chloroplast rearrangements. The leaf discs were kept continually moist by placement atop a wet filter paper strip, wicking water out of a reservoir, with a hole punched in the center as to not obstruct light transmission.
Leaf discs were subjected 2 h of 0.1 μmol photons m\(^{-2}\) s\(^{-1}\) of blue light, followed by 1 h of 30 μmol photons m\(^{-2}\) s\(^{-1}\) of blue light, and finally exposed to 100 μmol photons m\(^{-2}\) s\(^{-1}\) of blue light for 1 h. Transmission was measured minutely by turning off the blue actinic light for 100 μs and turning on the low intensity red light (<0.0124 μmol photons m\(^{-2}\) s\(^{-1}\)) to measure the percentage of red light transmitted through the leaf to the phototransistor located underneath the leaf.

Relating Nonphotochemical Quenching and Chloroplast Movement

In order to assess potential relationships between the capacities of plants for nonphotochemical quenching and chloroplast movement, we correlated our NPQ\(_{\text{max}}\) and stress recovery data with data collected by Königer and Bollinger (2012) describing the speed and degree to which E. crassipes, Hosta ‘Krossa Regal’, T. officinale, and A. thaliana WT and chup1 could relocate their chloroplasts in the avoidance and accumulation responses, which was assessed by measuring changes in the transmission of light through leaves (see previous section). Because Königer and Bollinger (2012) did not characterize chloroplast movement in A. thaliana npq1, we were unable to include that mutant in our comparisons. In addition, we compared our NPQ\(_{\text{max}}\) and stress recovery data to equivalent stress recovery data collected from leaves treated with the chloroplast movement inhibitor, cytochalasin B (CytB), from Kwon (2011).
RESULTS

In this study we sought to compare the photoprotective importance of nonphotochemical quenching across different plant species and *A. thaliana* mutants. We compared the ability of different plants to perform nonphotochemical quenching and quantified the role of nonphotochemical quenching in the recovery of leaves from high light stress for each species and mutant. Lastly, we related our results to previous data characterizing and quantifying the importance of chloroplast movement for the same species.

Evaluating the Effects of Leaf Disc Treatment and DTT on Photosynthesis

Assessing the Effect of Vacuum Infiltration on Stress Tolerance

In order to specifically elucidate the importance of nonphotochemical quenching in recovery from high light stress, the chemical inhibitor DTT was used to impair the function of violaxanthin de-epoxidase. Because the treatment of leaf discs with DTT involved the potentially damaging process of vacuum infiltration, it was important to assess the relevant impact of treatment on stress tolerance. Overall, leaf discs that were vacuum infiltrated with buffer recovered slightly less of their photosynthetic yield after exposure to 90 min of high light stress compared to untreated fresh leaves (Figure 1). There was a strong correlation between the ability of plants to recover from high light with treated and untreated leaf tissue, suggesting that vacuum infiltration does not disproportionately affect any particular species or genotype (Figure 1).
FIGURE 1. Comparison of the ability of fresh leaves and leaf tissue vacuum infiltrated with buffer to recover from high light stress. Fresh picked leaves or leaf discs treated with buffer of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT and *chup1* were acclimated to low light (LL, 10 μmol photons m$^{-2}$s$^{-1}$) for 30 min, followed by exposure to 90 min of high light (HL, 1000 μmol photons m$^{-2}$s$^{-1}$) and a final 60 min LL recovery period. The percent recovery was determined by comparing yield values, measured via chlorophyll $a$ fluorescence, at the end of the recovery period to those at the end of the initial LL acclimation period. Values shown are means for each species or genotype ($n$= 8-18). Lines of best fit are shown with correlations ($R^2$). Fresh leaf data were taken from Königer and Bollinger (2012).

Determining the Optimal Concentration of DTT

Kwon (2011) found that 30 mM DTT was sufficient to decrease the level of nonphotochemical quenching in *A. thaliana* WT to that of the *npq1* mutant. Because the species used in this study varied greatly in terms of leaf tissue thickness, we evaluated the efficacy of additional concentrations of DTT to ensure nonphotochemical quenching was adequately inhibited across species. While 30 mM and 40 mM DTT inhibited nonphotochemical quenching in *T. officinale* to the same degree, the 40 mM concentration had a greater effect on
nonphotochemical quenching than 30 mM DTT in *E. crassipes*, the species with the thickest leaves (Figure 2). Because the 30 mM concentration is insufficient to inhibit zeaxanthin-dependent nonphotochemical quenching in all species, 40 mM DTT was used in all following experiments.

![Graph](image)

**FIGURE 2. Comparison of the degree of inhibition of nonphotochemical quenching (NPQ) associated with different DTT concentrations.** Leaf discs of *E. crassipes* and *T. officinale* treated with 0 mM, 30 mM, or 40 mM DTT were irradiated with a 10 s pulse of far-red light and dark adapted for 10 min and subsequently exposed to high light (HL, 1000 µmol photons m⁻² s⁻¹) for 12 min, during which NPQ was measured at 1 min intervals via chlorophyll a fluorescence. Subsequent to the HL treatment, leaves were returned to dark conditions and minutely NPQ measurements were continued for 15 min. Values shown are mean ± SD (*n* = 3-7).

*Evaluating Non-Target Effects of DTT*

Because the reducing agent DTT is a nonspecific inhibitor, it was necessary to evaluate its impact on other photosynthetic measures relevant to this study. To assess the effect of DTT on the photosynthetic capacity of leaves, Fv/Fm, the maximum quantum photochemical efficiency, was measured in dark adapted leaves. Fv/Fm for buffer treated leaf discs ranged from
0.720 to 0.829, while Fv/Fm ranged from 0.662 to 0.792 in DTT treated tissue. While DTT treatment did not appear to drastically affect photosynthetic capacity, DTT-treated leaf discs had significantly lower Fv/Fm values than their buffer-treated counterparts for almost all species and genotypes (Table 3), indicating the DTT treatment does have a detrimental effect. The only plant for which DTT treatment did not quite result in a significant decrease was A. thaliana npq1, which had a nearly significant difference with a p-value of 0.0664 (Table 4). Because of the demonstrated pre-stress depressions in Fv/Fm associated with DTT treatment, it was important that relative measures were used in analyses of measurements based on photochemical efficiency. DTT treatment did not appear to disrupt chloroplast movement, as DTT-treated and -untreated leaves showed similar patterns of leaf transmission when subjected to increasing light intensities (data not shown). Preliminary experiments by Kwon (2011) also demonstrate that DTT does not have an effect on chloroplast movement.

**TABLE 3. The effects of DTT treatment on maximum quantum photochemical efficiency (Fv/Fm).** Leaf discs of *E. crassipes, Hosta ‘Krossa Regal’, T. officinale*, and *A. thaliana WT, npq1, and chup1* treated with 0 mM or 40 mM DTT were exposed to 10s of far-red light and dark adapted for 10 min, after which Fv/Fm was determined via chlorophyll a fluorescence. Fv/Fm values shown are means (± SD). T-tests were used to compare Fv/Fm values between treatments within each species/genotype (*n* = 4-7). Significant differences (*α* = 0.05) are indicated by asterisks (*) after p-values.

<table>
<thead>
<tr>
<th>Species/genotype</th>
<th>Fv/Fm (0 mM DTT)</th>
<th>Fv/Fm (40 mM DTT)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. crassipes</em></td>
<td>0.813 ± 0.012</td>
<td>0.775 ± 0.14</td>
<td>0.0040*</td>
</tr>
<tr>
<td>Hosta ‘Krossa Regal’</td>
<td>0.767 ± 0.023</td>
<td>0.707 ± 0.032</td>
<td>0.0032*</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>0.811 ± 0.008</td>
<td>0.763 ± 0.008</td>
<td>&lt;0.0001*</td>
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<td><em>A. thaliana WT</em></td>
<td>0.798 ± 0.007</td>
<td>0.757 ± 0.010</td>
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<tr>
<td><em>A. thaliana npq1</em></td>
<td>0.784 ± 0.043</td>
<td>0.740 ± 0.015</td>
<td>0.0664</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>0.794 ± 0.015</td>
<td>0.773 ± 0.012</td>
<td>0.0395*</td>
</tr>
</tbody>
</table>
Comparing Nonphotochemical Quenching between Species and Mutants

The Kinetics and Capacity of Nonphotochemical Quenching

In order to compare the abilities of different species to perform nonphotochemical quenching, we examined the nonphotochemical quenching kinetics of leaf discs exposed to 12 min of high light stress (Figure 3, white circles and dotted lines). The amount of nonphotochemical quenching increased in all species and *A. thaliana* genotypes upon exposure to high light with greatest increases occurring during the first 2 min of exposure to high light, after which nonphotochemical quenching began to level off for most plants (Figure 3). Nonphotochemical quenching relaxed upon the return to dark conditions in all species. The most dramatic drop in nonphotochemical quenching occurred within the first minute of the dark recovery period for all plants, after which relaxation slowed and eventually reached a constant level (Figure 3). Despite this general pattern, species varied in both the kinetics and degree to which nonphotochemical quenching was performed. Both the induction and relaxation of nonphotochemical quenching occurred most rapidly in *T. officinale*, which also achieved the highest level of total nonphotochemical quenching. In contrast, the kinetics were the most gradual in *Hosta ‘Krossa Regal’*, which had the slowest induction and relaxation of nonphotochemical quenching, and achieved one of the lowest levels of total nonphotochemical quenching (Figures 3 & 4).
FIGURE 3. Comparison of nonphotochemical quenching (NPQ) kinetics and DTT inhibition across species and genotypes. Leaf discs of *E. crassipes*, *Hosta 'Krossa Regal'*, *T. officinale*, and *A. thaliana* WT, npq1, and chup1 treated with 0 mM or 40 mM DTT were irradiated with a 10 s pulse of far-red light and dark adapted for 10 min and subsequently exposed to high light (HL, 1000 µmol photons m⁻² s⁻¹) for 12 min, during which NPQ was measured at 1 min intervals via chlorophyll a fluorescence. Subsequent to the HL treatment, leaves were returned to dark conditions and minutely NPQ measurements were continued for 15 min. Values shown are means ± SD (n= 4-7).
Species exhibited different capacities to perform nonphotochemical quenching, as the maximum level of nonphotochemical quenching during the 12 min high light treatment (NPQ$_{\text{max}}$) varied between plants (Figure 4). *T. officinale* had the greatest capacity for nonphotochemical quenching, followed by *A. thaliana chup1*, *E. crassipes*, wild type *A. thaliana*, and *Hosta ‘Krossa Regal’* (Figure 4). As expected, the violaxanthin de-epoxidase deficient mutant, *A. thaliana npq1*, exhibited the lowest capacity for nonphotochemical quenching, though its mean NPQ$_{\text{max}}$ was surprisingly not significantly different than *A. thaliana* WT (Table 4).

Treatment with 40 mM DTT significantly impaired the ability of all plants to perform nonphotochemical quenching, except for the *A. thaliana npq1* mutant, which inherently exhibits impaired nonphotochemical quenching function (Table 4). Treatment with 40mM DTT decreased the capacity of *A. thaliana* WT and *chup1* to a level similar to that exhibited by the *npq1* mutant, suggesting that the inhibitor concentration used is sufficient to suppress violaxanthin de-depoxidase (Figure 4, Table 4). All species exhibited attenuated, but not completely diminished, capacities for nonphotochemical quenching when treated with DTT, suggesting that all species utilize zeaxanthin-independent nonphotochemical quenching to some degree (Figures 3 & 4). DTT treatment most greatly reduced nonphotochemical quenching in *E. crassipes*, resulting in a 71% reduction in NPQ$_{\text{max}}$, followed by *T. officinale*, *A. thaliana chup1*, *Hosta ‘Krossa Regal’*, and *A. thaliana* WT, which suffered 60%, 45%, 44%, and 40% reductions, respectively (Figure 4). *A. thaliana npq1*, the only plant for which DTT treatment did not significantly reduce NPQ$_{\text{max}}$, only suffered a 11% depression in total nonphotochemical quenching capacity (Figure 4, Table 4).

Despite differences in the percent reductions of nonphotochemical quenching capacity between species and genotypes, DTT treatment equalized nonphotochemical quenching between
the different plant types. Subsequent to DTT treatment, all A. thaliana genotypes exhibited similar \( \text{NPQ}_{\text{max}} \) values, whereas without DTT treatment the \textit{chup}1 mutant had a significantly greater capacity for nonphotochemical quenching than the \textit{npq}1 mutant (Table 4). Likewise, nonphotochemical quenching capacities of \textit{T. officinale}, \textit{Hosta ‘Krossa Regal’}, and all of the \textit{A. thaliana} genotypes were indistinguishable after treatment with DTT, whereas significant differences had existed between species previously (Table 4). The only differences in \( \text{NPQ}_{\text{max}} \) post-DTT treatment were observed in \textit{E. crassipes}, which had a significantly lower capacity for nonphotochemical quenching compared to \textit{A. thaliana npq}1 and \textit{chup}1 (Table 4).

FIGURE 4. Comparison of mean (± SD) maximum value of nonphotochemical quenching (\( \text{NPQ}_{\text{max}} \)) across species and genotypes in DTT-treated and -untreated leaves. Leaf discs of \textit{E. crassipes}, \textit{Hosta ‘Krossa Regal’}, \textit{T. officinale}, and \textit{A. thaliana} WT, \textit{npq}1, and \textit{chup}1 treated with 0 mM or 40 mM DTT were irradiated with a 10 s pulse of far-red light and dark adapted for 10 min and subsequently exposed to high light (HL, 1000 \( \mu \text{mol} \text{ photons m}^{-2} \text{s}^{-1} \)) for 12 min, during which NPQ was measured at 1 min intervals via chlorophyll \( \alpha \) fluorescence. \( \text{NPQ}_{\text{max}} \) represents the greatest NPQ value achieved during the HL treatment (see Figure 3) \( (n=4-7) \).
TABLE 4. Statistical comparison of maximum value of nonphotochemical quenching (NPQ$_{\text{max}}$) across species and genotypes in DTT-treated and -untreated leaves. Leaf discs of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT, npq1, and chup1 treated with 0 mM or 40 mM DTT were irradiated with a 10 s pulse of far-red light and dark adapted for 10 min and subsequently exposed to high light (HL, 1000 µmol photons m$^{-2}$s$^{-1}$) for 12 min, during which NPQ was measured at 1 min intervals via chlorophyll *a* fluorescence. NPQ$_{\text{max}}$ represents the greatest NPQ value achieved during the HL treatment (*n* = 4-7) (see Figure 3) and was compared between treatments and across species and genotypes via two-way ANOVA and Tukey-Kramer HSD tests. Entries not connected by the same letter are significantly different.

<table>
<thead>
<tr>
<th>Species/genotype</th>
<th>DTT treatment</th>
<th>Tukey level</th>
<th>Mean NPQ$_{\text{max}}$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. officinale</em></td>
<td>0 mM</td>
<td>A</td>
<td>3.02 ± 0.30</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>0 mM</td>
<td>A B</td>
<td>2.54 ± 0.16</td>
</tr>
<tr>
<td><em>E. crassipes</em></td>
<td>0 mM</td>
<td>B C</td>
<td>2.42 ± 0.24</td>
</tr>
<tr>
<td><em>A. thaliana</em> WT</td>
<td>0 mM</td>
<td>B C D</td>
<td>2.06 ± 0.23</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>0 mM</td>
<td>C D E</td>
<td>1.96 ±0.10</td>
</tr>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>0 mM</td>
<td>D E F</td>
<td>1.66 ± 0.26</td>
</tr>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>40 mM</td>
<td>E F G</td>
<td>1.49 ± 0.23</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>40 mM</td>
<td>F G</td>
<td>1.40 ± 0.15</td>
</tr>
<tr>
<td><em>A. thaliana</em> WT</td>
<td>40 mM</td>
<td>F G H</td>
<td>1.23 ± 0.12</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>40 mM</td>
<td>F G H</td>
<td>1.20 ± 0.11</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>40 mM</td>
<td>G H</td>
<td>1.10 ± 0.27</td>
</tr>
<tr>
<td><em>E. crassipes</em></td>
<td>40 mM</td>
<td>H</td>
<td>0.71 ± 0.17</td>
</tr>
</tbody>
</table>
While most nonphotochemical quenching induced under high light relaxed upon exposure to dark conditions, all species continued to thermally dissipate energy 15 min after return to darkness (Figures 3 & 4). Because of the slow reversibility of this nonphotochemical quenching, it was deemed ‘sustained nonphotochemical quenching’. Sustained nonphotochemical quenching varied between species with the *A. thaliana* mutants, *npq1* and *chup1*, exhibiting the greatest amount of thermal quenching in the dark, followed by *Hosta ‘Krossa Regal’*, *A. thaliana* WT, *E. crassipes*, and *T. officinale*, respectively (Figure 5, Table 5). The *A. thaliana npq1* mutant performed significantly more sustained nonphotochemical quenching than wild type *A. thaliana*, *E. crassipes*, and *T. officinale*. Treatment of leaf discs with DTT did not significantly affect the level of sustained dissipation (Figure 5, Table 5).

**FIGURE 5.** Comparison of mean (± SD) value of sustained nonphotochemical quenching (NPQ) across species and genotypes in DTT-treated and -untreated leaves. Leaf discs of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT, *npq1*, and *chup1* treated with 0 mM or 40 mM DTT were irradiated with a 10 s pulse of far-red light and dark adapted for 10 min and subsequently exposed to high light (HL, 1000 µmol photons m\(^{-2}\)s\(^{-1}\)) for 12 min. Subsequent to the HL treatment, leaves were exposed to another 10 s pulse of far-red light and returned to dark conditions for a 15 min recovery period. The final NPQ value measured at the end of the recovery period via chlorophyll a fluorescence was deemed sustained NPQ (*n* = 4-7).
### TABLE 5. Statistical comparison of sustained nonphotochemical quenching across species and genotypes in DTT-treated and -untreated leaves.

Leaf discs of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT, *npq1*, and *chup1* treated with 0 mM or 40 mM DTT were irradiated with a 10 s pulse of far-red light and dark adapted for 10 min and subsequently exposed to high light (HL, 1000 µmol photons m\(^{-2}\)s\(^{-1}\)) for 12 min. Subsequent to the HL treatment, leaves were exposed to another 10 s pulse of far-red light and returned to dark conditions for a 15 min recovery period (see Figure 1). Sustained NPQ represents the final NPQ value measured at the end of the recovery period via chlorophyll \(a\) fluorescence \((n=4-7)\) and was compared between treatments and across species and genotypes via two-way ANOVA and Tukey-Kramer HSD tests. Entries not connected by the same letter are significantly different.

<table>
<thead>
<tr>
<th>Species/genotype</th>
<th>DTT treatment</th>
<th>Tukey level</th>
<th>Mean sustained NPQ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>40 mM</td>
<td>A</td>
<td>0.92 ± 0.20</td>
</tr>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>0 mM</td>
<td>A</td>
<td>0.86 ± 0.09</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>40 mM</td>
<td>A B</td>
<td>0.82 ± 0.11</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>0 mM</td>
<td>A B C</td>
<td>0.71 ± 0.15</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>40 mM</td>
<td>A B C</td>
<td>0.64 ± 0.14</td>
</tr>
<tr>
<td><em>A. thaliana WT</em></td>
<td>40 mM</td>
<td>A B C</td>
<td>0.56 ± 0.05</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>0 mM</td>
<td>A B C</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>40mM</td>
<td>C</td>
<td>0.48 ± 0.17</td>
</tr>
<tr>
<td><em>E. crassipes</em></td>
<td>0 mM</td>
<td>B C</td>
<td>0.46 ± 0.28</td>
</tr>
<tr>
<td><em>A. thaliana WT</em></td>
<td>0 mM</td>
<td>C</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td><em>E. crassipes</em></td>
<td>40 mM</td>
<td>C</td>
<td>0.37 ± 0.23</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>0 mM</td>
<td>C</td>
<td>0.36 ± 0.19</td>
</tr>
</tbody>
</table>
In order to compare the degree to which the different plants were able to utilize absorbed light for photosynthesis under high light stress, we also determined the coefficient of photochemical fluorescence quenching (qP) after leaves had been exposed to 12 min of high light (Figure 6). qP was similar between most species and genotypes, except for *T. officinale*, which was able to quench significantly greater amounts of fluorescence via photochemistry than any other plant. *T. officinale* was also the only species for which DTT-treatment significantly reduced qP (Figure 6).

**FIGURE 6.** Comparison of mean (± SD) value of photochemical quenching (qP) across species and genotypes in DTT-treated and -untreated leaves. Leaf discs of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT, *npq1*, and *chup1* treated with 0 mM or 40 mM DTT were irradiated with a 10 s pulse of far-red light and dark adapted for 10 min. Leaf discs were subsequently exposed to high light (HL, 1000 µmol photons m⁻² s⁻¹) for 12 min, after which qP was determined via chlorophyll a fluorescence (*n*= 4-7). Means were compared between treatments and across species and genotypes via two-way ANOVA tests. Significant differences (*α* = 0.05) are indicated by asterisks (*) after p-values.
The Role of Nonphotochemical Quenching in Recovery from High Light Stress

In order to isolate the photoprotective importance of nonphotochemical quenching for each plant, we tracked the photosynthetic yield of DTT-treated and -untreated leaves during high light stress treatments using chlorophyll a fluorescence (Figure 7). During the initial low light acclimation period, most plants exhibited yield values of about 0.7, indicating that neither the environmental conditions nor the preceding treatment caused severe stress (Figure 7). Although treatment with DTT did not dramatically reduce the photosynthetic capacity of leaf discs, most species exhibited significantly lower pre-stress yields when treated with DTT (Table 6). The only two plants for which DTT treatment did not significantly affect initial yield were Arabidopsis thaliana chup1 and Hosta ‘Krossa Regal’. All species exhibited indistinguishable yields prior to the stress treatment in the absence of DTT, except for Hosta ‘Krossa Regal’, which had a significantly lower pre-stress yield (Table 6). Because of the observed variation in photosynthetic yield prior to high light exposure, it was important to use relative measurements in comparing photosynthetic efficiency between species and treatments.

All leaves, regardless of species, genotype, or treatment, suffered dramatic depressions in photosynthetic yield during the 90 min period of exposure to high light (1000 µmol photons m\(^{-2}\)s\(^{-1}\)) compared to the initial low light acclimation period (10 µmol photons m\(^{-2}\)s\(^{-1}\)), dropping from unstressed pre-stress values of about 0.7 to yields below 0.2, which were sustained throughout the high light period (Figure 7). Upon the return to low light conditions, yields immediately improved in all plants, albeit to various degrees. While plants continued to recover their photochemical efficiency slowly throughout the 60 min recovery period, the bulk of recovered yield returned in the first 10 min, after which increases in yield began to saturate (Figure 7).
All species and genotypes displayed similar abilities to recover from high light stress when nonphotochemical quenching function was uninhibited (Figure 8, Table 7). Wild type *A. thaliana* exhibited the greatest resilience to light stress, recovering 88% of photosynthetic yield 1 h after return to low light conditions, followed by *T. officinale*, *E. crassipes*, *A. thaliana npq1*, and *Hosta ‘Krossa Regal’*, respectively, all of which recovered more than 80% of their pre-stress photochemical efficiency (Figure 8). The *chup1* mutant of *A. thaliana* was the least resilient to light stress, recovering only 72% of its original photosynthetic yield, though its recovery was not significantly lower than that of the other plants (Table 7).

Despite displaying similar abilities to recover from light stress when nonphotochemical quenching was uninhibited, species exhibited a greater range of resilience to high light after treatment with DTT (Figures 7 & 8). The only species for which DTT treatment did not significantly affect recovery from light stress was *E. crassipes*, which only suffered a 10% decrease in recovery associated with DTT treatment (Table 7). There was significant variation among the species for which DTT treatment affected recovery (Table 7). The three *A. thaliana* genotypes exhibited the least resilience to high light stress when treated with DTT, recovering significantly less than any of the other species (Figure 8, Table 7). *A. thaliana* WT and *npq1* in particular displayed the most dramatically attenuated capacities to recover, both suffering decreases in recovery of greater than 50%. *T. officinale* and *Hosta ‘Krossa Regal’* both recovered 65% of their pre-stress photochemical efficiency when treated with DTT, a more than 20% decrease compared to when their leaves were not treated with DTT (Figure 8).
FIGURE 7. Comparison of the effects of a high light stress treatment on the photosynthetic yield of leaves with inhibited and uninhibited nonphotochemical quenching capacity across species and genotypes. Leaf discs of E. crassipes, Hosta ‘Krossa Regal’, T. officinale, and A. thaliana WT, npq1, and chup1 treated with 0 mM or 40 mM DTT were acclimated to low light (LL, 10 µmol photons m$^{-2}$s$^{-1}$) for 30 min, followed by 90 min of high light (HL, 1000 µmol photons m$^{-2}$s$^{-1}$) and a final 60 min LL recovery period. The effective quantum yield of photosystem II was measured every 10 min during the LL periods and every 30 min during the HL treatment via chlorophyll a fluorescence. Yields shown are mean values ± SD (n= 8-18).
**TABLE 6. Statistical comparison of photosynthetic yield under low light conditions between treatments and across species and genotypes.** Leaf discs of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT, *npq1*, and *chup1* treated with 0 mM or 40 mM DTT were acclimated to low light (LL, 10 µmol photons m\(^{-2}\)s\(^{-1}\)) for 30 min, after which the effective quantum yield of photosystem II was measured via chlorophyll \(a\) fluorescence. Yields were compared between treatments and across species and genotypes via two-way ANOVA and Tukey-Kramer HSD tests \((n= 8\text{-}18)\). Entries not connected by the same letter are significantly different.

<table>
<thead>
<tr>
<th>Species/genotype</th>
<th>DTT treatment</th>
<th>Tukey level</th>
<th>Mean yield ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. crassipes</em></td>
<td>0 mM</td>
<td>A</td>
<td>0.77 ± 0.02</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>0 mM</td>
<td>A</td>
<td>0.75 ± 0.01</td>
</tr>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>0 mM</td>
<td>A</td>
<td>0.75 ± 0.02</td>
</tr>
<tr>
<td><em>A. thaliana WT</em></td>
<td>0 mM</td>
<td>A</td>
<td>0.75 ± 0.02</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>0 mM</td>
<td>A</td>
<td>0.74 ± 0.01</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>40 mM</td>
<td>B</td>
<td>0.71 ± 0.01</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>0 mM</td>
<td>C</td>
<td>0.71 ± 0.03</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>40mM</td>
<td>D</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td><em>E. crassipes</em></td>
<td>40 mM</td>
<td>D</td>
<td>0.68 ± 0.02</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>40 mM</td>
<td>D</td>
<td>0.67 ± 0.01</td>
</tr>
<tr>
<td><em>A. thaliana WT</em></td>
<td>40 mM</td>
<td>E</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>40 mM</td>
<td>E</td>
<td>0.65 ± 0.04</td>
</tr>
</tbody>
</table>
FIGURE 8. Comparison of mean (± SD) percent recovery of photosynthetic yield of high light stressed leaves with inhibited and uninhibited nonphotochemical quenching capacity across species and genotypes. Leaf discs of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT, *npq1*, and *chup1* treated with 0 mM or 40 mM DTT were acclimated to low light (LL, 10 µmol photons m⁻² s⁻¹) for 30 min, followed by exposure to 90 min of high light (HL, 1000 µmol photons m⁻² s⁻¹) and a final 60 min LL recovery period. The percent recovery was determined by comparing yield values, determined via chlorophyll *a* fluorescence, at the end of the recovery period to those at the end of the initial LL acclimation period (*n* = 8-18).
TABLE 7. Statistical comparison of percent recovery of photosynthetic yield of high light stressed leaves with inhibited and uninhibited nonphotochemical quenching capacity across species and genotypes. Leaf discs of *E. crassipes*, *Hosta ‘Krossa Regal’*, *T. officinale*, and *A. thaliana* WT, npq1, and chup1 treated with 0 mM or 40 mM DTT were acclimated to low light (LL, 10 µmol photons m\(^{-2}\)s\(^{-1}\)) for 30 min, followed by exposure to 90 min of high light (HL, 1000 µmol photons m\(^{-2}\)s\(^{-1}\)) and a final 60 min LL recovery period (see Figure 7). The percent recovery was determined by comparing yield values, determined via chlorophyll \(a\) fluorescence, at the end of the recovery period to those at the end of the initial LL acclimation period (\(n=8-18\)) and was compared between treatments and across species and genotypes via two-way ANOVA and Tukey-Kramer HSD tests. Entries not connected by the same letter are significantly different.

<table>
<thead>
<tr>
<th>Species/genotype</th>
<th>DTT treatment</th>
<th>Tukey level</th>
<th>Mean % recovery ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. thaliana</em> WT</td>
<td>0 mM</td>
<td>A</td>
<td>87.7 ± 4.1</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>0 mM</td>
<td>A</td>
<td>87.4 ± 6.6</td>
</tr>
<tr>
<td><em>E. crassipes</em></td>
<td>0 mM</td>
<td>A</td>
<td>85.7 ± 5.8</td>
</tr>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>0 mM</td>
<td>A</td>
<td>81.5 ± 14.3</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>0 mM</td>
<td>A</td>
<td>81.3 ± 4.4</td>
</tr>
<tr>
<td><em>E. crassipes</em></td>
<td>40 mM</td>
<td>A</td>
<td>75.7 ± 11.0</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>0 mM</td>
<td>A, B</td>
<td>72.1 ± 9.2</td>
</tr>
<tr>
<td><em>Hosta ‘Krossa Regal’</em></td>
<td>40 mM</td>
<td>B</td>
<td>64.9 ± 5.6</td>
</tr>
<tr>
<td><em>T. officinale</em></td>
<td>40 mM</td>
<td>B</td>
<td>64.6 ± 9.9</td>
</tr>
<tr>
<td><em>A. thaliana chup1</em></td>
<td>40 mM</td>
<td>C</td>
<td>43.0 ± 13.2</td>
</tr>
<tr>
<td><em>A. thaliana WT</em></td>
<td>40 mM</td>
<td>C</td>
<td>36.8 ± 15.5</td>
</tr>
<tr>
<td><em>A. thaliana npq1</em></td>
<td>40 mM</td>
<td>C</td>
<td>31.4 ± 15.6</td>
</tr>
</tbody>
</table>
The Relationship between Nonphotochemical Quenching and Stress Tolerance

The capacity of plants to perform nonphotochemical quenching was not clearly associated with the resilience to high light stress (Figure 9). Plants that achieved higher NPQ\textsubscript{max} values did not necessarily show an enhanced ability to recover from high light exposure (Figure 9, white circles). Interestingly, when zeaxanthin-dependent nonphotochemical quenching was inhibited with DTT, NPQ\textsubscript{max} was negatively correlated with recovery from high light stress (Figure 9, black squares).

**FIGURE 9.** The relationship between maximum nonphotochemical quenching (NPQ\textsubscript{max}) and recovery from high light stress. NPQ\textsubscript{max} and percent recovery values were determined for *E. crassipes*, *Hosta 'Krossa Regal'* , *T. officinale*, and *A. thaliana* WT, *npq1*, and *chup1* leaves treated with and without DTT (Figures 4 & 8). Data points represent mean value for each species/genotype and treatment (n= 4-18). Lines of best fit are shown with correlations (R\textsuperscript{2}).
Relating Nonphotochemical Quenching and Chloroplast Movement

In order to examine the potential relationship between the two photoprotective strategies, nonphotochemical quenching and chloroplast movement, we compared our data describing the capacity of plants to perform nonphotochemical quenching with data characterizing chloroplast movement for the same species collected by Königer and Bollinger (2012). Königer and Bollinger (2012) characterized chloroplast movement through changes in leaf transmission during exposure to different intensities of blue light, ranging from dark to 100 µmol photons m$^{-2}$s$^{-1}$, and calculated the maximum speed of both the chloroplast avoidance and accumulation responses in terms of maximum percent change in transmission per hour. They also determined the maximum degree of chloroplast avoidance and accumulation behaviors in terms of the maximum percent change in transmission during each response. Because they did not study the A. thaliana npq1 mutant, we were only able to compare the five other plants: E. crassipes, Hosta ‘Krossa Regal’, T. officinale, and A. thaliana WT and chup1. We did not observe a strong relationship between NPQ$_{\text{max}}$ and the maximum speed with which plants were able to relocate their chloroplasts in the avoidance response (Figure 10A), though there was a slight negative correlation between NPQ$_{\text{max}}$ and the degree to which plants could change their chloroplast arrangement in the avoidance response (Figure 10C). Similarly, NPQ$_{\text{max}}$ was not strongly correlated with either the maximum speed or degree of to which plants could move their chloroplasts in the accumulation response (Figure 10B & D).
FIGURE 10. The relationship between the capacities of plants for nonphotochemical quenching and chloroplast movement. Comparisons of maximum nonphotochemical quenching (NPQ\text{max}) (see Figure 4) and the maximum speed of chloroplast movement in the avoidance (A) and accumulation (B) responses expressed as percent change in leaf transmission per hour when exposed to increasing light intensities. NPQ\text{max} was also compared with the maximum degree of chloroplast avoidance (C) and accumulation (D) responses, expressed as percent change in leaf transmission. Data shown are averages for E. crassipes, Hosta ‘Krossa Regal’, T. officinale, and A. thaliana WT and chup1 (n = 4-8). Chloroplast movement data were taken from Königer and Bollinger (2012). Lines of best fit are shown with correlations (R²).
In order to further examine the potential relationship between nonphotochemical quenching and chloroplast movement, we compared the abilities of plants to recover from high light stress when each of the two photoprotective processes were individually inhibited, specifically relating the observed recoveries of the inhibited plants to the capacities of the plants to perform nonphotochemical quenching and the chloroplast avoidance response. Interestingly, plants that were more facile chloroplast movers did not display an enhanced ability to recover from high light stress when their capacity for nonphotochemical quenching was inhibited with DTT (Figure 11A & B). In fact, the percent recovery of photosynthetic yield of DTT-treated leaves was slightly negatively correlated with both the speed and degree of the chloroplast movement avoidance response (Figure 11A & B). The *A. thaliana chup1* mutant, which displayed the most limited ability to relocate its chloroplasts, appeared to fit least well with the data points of the other plants (Figure 11A & B).

In order to compare the ability of plants to perform nonphotochemical quenching and recover from high light exposure in the absence of chloroplast movement, we compared $\text{NPQ}_{\text{max}}$ with the ability of each species to recover when treated with cytochalasin B (CytB), an actin depolymerizing agent that eliminates the ability of plants to reposition their chloroplasts. Percent recovery data for CytB-treated leaf discs was taken from Kwon (2011). Nonphotochemical quenching capacity and recovery from light stress under CytB treatment were weakly positively correlated (Figure 11C). Interestingly, species and mutants that were better able to recover from light stress in the absence of chloroplast movement also showed enhanced abilities to recover when their capacity for nonphotochemical quenching was selectively inhibited (Figure 11D). Once again, the *chup1* mutant tended to be an outlier (Figure 11C & D).
FIGURE 11. Relationship between the photoprotective capacities of plants and their ability to recover from high light stress with inhibited photoprotective function.

Comparison of the ability of plants treated with DTT to recover from high light stress (see Figure 8) and the (A) speed and (B) degree to which they are able to move their chloroplasts in the avoidance response, expressed as percent change in transmission and percent change in transmission per hour, respectively. Chloroplast movement data were taken from Königer and Bollinger (2012). The ability of plants treated with CytB (chloroplast movement inhibitor) to recover from high light stress was compared to (C) the capacity of each plant to perform nonphotochemical quenching (NPQ\textsubscript{max}) (see Figure 4) and (D) the ability of plants to recover from high light stress when treated with DTT. CytB-treated percent recoveries were taken from Kwon (2011). Data shown are averages for E. crassipes, Hosta ‘Krossa Regal’, T. officinale, and A. thaliana WT, npq1, and chup1 (n= 4-8), except (A) and (B) do not include data for the npq1 mutant. Lines of best fit are shown with correlations (R\textsuperscript{2}). Data points representing the chup1 mutant are indicated with arrows.
DISCUSSION

In this study we sought to investigate the role that nonphotochemical quenching plays in the recovery of plants from high light exposure with the ultimate goal of comparing the relative photoprotective importance of nonphotochemical quenching and chloroplast movement. Overall, we found that species differed in the capacity to which they could perform nonphotochemical quenching, but that those plants with greater abilities to dissipate excess energy as heat did not necessarily demonstrate enhanced resilience to high light stress. Interestingly, there did not appear to be a relationship between the abilities of plants to perform nonphotochemical quenching and light-induced chloroplast movements.

Nonphotochemical Quenching Capacity Varies between Species

Despite displaying similar patterns of nonphotochemical quenching kinetics, the different species and mutants observed in this study performed nonphotochemical quenching to different degrees, exhibiting nonphotochemical quenching capacities ranging from 1.66 to 3.02 (Figure 4). Species have been observed to exhibit maximum values of nonphotochemical quenching ranging from 1 to 5 in their natural environments (Adams et al. 1989; Bilger et al. 1995; Demmig-Adams 1998; Demmig-Adams and Adams 1996; Johnson et al. 1993). *T. officinale,* which demonstrated the greatest overall capacity for nonphotochemical quenching, also induced nonphotochemical quenching the most quickly (Figure 3). *T. officinale* may have a greater xanthophyll pigment pool (violaxanthin + antheraxanthin + zeaxanthin) size than the other plants, as plants with more extensive xanthophyll pigment pools have been observed to induce thermal dissipation to a greater degree than their counterparts with less pigments to convert to the dissipative state (Demmig-Adams 1998; Johnson et al. 2008). For example, *Aquilegia coerulea,* which has a xanthophyll carotenoid pool of 118 mmol per mol of chlorophyll, has been observed to have a
maximum nonphotochemical quenching value of 5.0, while *Euonymus kiautschovicus*, which has a xanthophyll pool of only 39 mmol per mol of chlorophyll, was only observed to have a maximum nonphotochemical quenching value of 1.4 (Demmig-Adams 1998). *T. officinale* is also a weedy plant that naturally grows well in sunny areas and plants that grow in high light environments tend to have larger xanthophyll pigment pools than their shade-dwelling counterparts (Demmig-Adams 1998; Golan et al. 2006; Kato et al. 2003; König et al. 1995).

Consistent with this pattern, the shade plant *Hosta ‘Krossa Regal’* thrives in low light environments and had the lowest capacity for nonphotochemical quenching of all of the plants tested, with the exception of the *A. thaliana npq1* mutant (Figure 4).

It is also important to note that *T. officinale* leaves were collected from naturally-growing plants outdoors, while all other plants were grown under controlled constant light conditions. Thus, *T. officinale* was the only species exposed to naturally fluctuating light conditions and the fact that the benefits of nonphotochemical quenching become particularly pronounced under variable light (Külheim et al. 2002) may partially explain why *T. officinale* exhibited the greatest capacity for thermal dissipation.

After *T. officinale*, the *chup1* mutant of *A. thaliana* demonstrated the greatest capacity for nonphotochemical quenching (Figure 4). It is interesting that this mutant, which is virtually unable to perform normal chloroplast movement behavior (Oikawa et al. 2003), displayed a great ability to perform nonphotochemical quenching. Although the nonphotochemical quenching capacity of *A. thaliana chup1* was not significantly greater than that of its wild type counterpart (Table 4), perhaps the slight enhancement in nonphotochemical quenching capacity is one way that the mutant can compensate for its deficiencies in performing chloroplast movement. Despite not being able to reposition its chloroplasts, the *chup1* mutant was still able to recover 72% of its
pre-stress photochemical efficiency (Figure 8), a percentage that was not statistically different from that of wild type *A. thaliana* (Table 7), suggesting that *chup1* must be able to compensate for its inability to perform the chloroplast movement avoidance response to some extent.

As expected, the *A. thaliana npq1* mutant, deficient in the key xanthophyll cycle enzyme, violaxanthin de-epoxidase, exhibited the lowest capacity to perform nonphotochemical quenching (Figure 4). Despite this deficiency, the *npq1* mutant was still able to induce a modest amount of nonphotochemical quenching (Figure 4), which was surprisingly not significantly lower than that induced by wild type *A. thaliana* (Table 4). The lack of a significant difference between the NPQ<sub>max</sub> values of *A. thaliana* WT and *npq1*, as well as the general similarity of NPQ<sub>max</sub> values between the other species studied, may be partially attributed to the light conditions under which our experimental plants were grown. All of the *A. thaliana* plants, as well as *Hosta ‘Krossa Regal’*, were raised under constant conditions with relatively low light intensity, 170 μmol photons m\(^{-2}\)s\(^{-1}\), less than a tenth of the intensity of full sunlight (approximately 2000 μmol photons m\(^{-2}\)s\(^{-1}\)). *E. crassipes*, a species that thrives in higher light environments, was grown under 400 μmol photons m\(^{-2}\)s\(^{-1}\) of light, only 20% of the intensity of full sun. Thus, the plants used in this study are not acclimated to high light and may have smaller pools of xanthophyll carotenoids than if they were regularly challenged with high light.

Li et al. (2000), who grew their plants in naturally lit greenhouses, found wild type *A. thaliana* was able to induce more than three times as much nonphotochemical quenching after only 6 min of high light exposure, compared to the *npq1* mutant, while in our experiments we only observed a 1.2-fold difference in the nonphotochemical quenching capacities of these two genotypes (Figure 4). Although Li et al. (2000) exposed their leaves to a higher light intensity
during their nonphotochemical quenching measurements (1250 μmol photons m$^{-2}$s$^{-1}$), it seems unlikely that that difference alone would account for the comparatively dramatic difference in nonphotochemical quenching capacity that they observed between the two genotypes. Niyogi et al. (1998), who also grew their plants in naturally lit greenhouses, saw a nearly four-fold difference in the nonphotochemical quenching capacity between the wild type and npq1 genotypes of A. thaliana, though the magnitude of this difference may be partially attributed to the fact that they exposed their leaves to almost twice as high of a light intensity to induce nonphotochemical quenching during their measurements. It would be interesting to see if differences in photoprotective traits, particularly nonphotochemical quenching capacity, became more pronounced between the different species and A. thaliana genotypes that we studied if the plants were grown under higher light conditions.

It has also been widely recognized that zeaxanthin is not the only pigment responsible for nonphotochemical quenching (Johnson et al. 2009; Li et al. 2009; Pogson et al. 1998) and previous studies have shown that the A. thaliana npq1 mutant is still able to induce nonphotochemical quenching, albeit to a lesser extent than A. thaliana genotypes with unimpaired violaxanthin de-epoxidase function (Li et al. 2000; Niyogi et al. 1998). Much of zeaxanthin-independent nonphotochemical quenching has been attributed to the structurally similar pigment lutein (Li et al. 2009). Lutein is thought to be involved in the rapidly reversible component of nonphotochemical quenching, though its exact role has yet to be fully defined (Li et al. 2009; Pogson et al. 1998). The fact that npq1 mutants are not deficient in lutein production (Li et al. 2009) may explain why the npq1 mutants in our study were still able to perform nonphotochemical quenching, as well as why DTT treatment did not completely eliminate the capacity of any species or genotype to induce thermal dissipation (Figure 4).
In addition to exhibiting zeaxanthin-independent nonphotochemical quenching (Figure 4), all species also exhibited sustained nonphotochemical quenching that failed to relax after the leaves were returned to dark conditions (Figure 5). This sustained nonphotochemical quenching may be attributed to \textit{de novo} zeaxanthin synthesis (qZ) or photoinhibition (qI), rather than qE, which is rapidly reversible. The high Fv/Fm values of all plants prior to high light exposure suggest that the sustained quenching is not the result of prior photoinhibition (Table 3). The two plants that exhibited the greatest amount of sustained quenching, \textit{A. thaliana npq1} and \textit{chup1} mutants (Figure 5), may have been inherently more susceptible to photodamage due to their photoprotection-related mutations.

**Nonphotochemical Quenching Is Not the Only Important Photoprotective Mechanism**

While all species displayed similar abilities to recover from high light stress when their photoprotective processes were intact, treatment with DTT affected the abilities of species to recover to different degrees (Figure 8). DTT treatment did not significantly attenuate the resilience of \textit{E. crassipes} and \textit{Hosta ‘Krossa Regal’} to recover from light stress (Table 7), suggesting that nonphotochemical quenching may not have substantial photoprotective importance in these species grown under the light conditions used in this study. \textit{T. officinale} demonstrated a decreased ability to recover from light stress, but was still able to recover 65% of its pre-stress photosynthetic yield (Figure 7), indicating that nonphotochemical quenching was photoprotectively important, but was not the only photoprotective strategy that the species employed.

The three \textit{A. thaliana} genotypes were most severely affected by DTT treatment, suffering significantly greater decreases in their light stress resilience compared to the other plants (Table
All three genotypes showed similar abilities to recover from light stress when treated with DTT. It was surprising that the npq1 mutant, our negative control for which DTT did not have an effect on nonphotochemical quenching, showed a severely attenuated capacity to recover from high light stress when treated with DTT (Table 7). This suggests that DTT may be disrupting other processes in addition to nonphotochemical quenching.

DTT is a nonspecific reducing agent that has been used to inhibit the activity of violaxanthin de-epoxidase in many previous studies (Adams et al. 1990; Bilger and Björkman 1990; Demmig-Adams et al. 1990; Johnson et al. 2008; Niyogi et al. 1998; Winter and Königer 1989) and is not thought to adversely affect photosynthetic yield (Bailey and Walker 1992). However, most other studies treated leaves with lower concentrations of DTT, around 1-5 mM, while we treated leaves with 40 mM DTT (Adams et al. 1990; Bilger and Björkman 1990; Demmig-Adams et al. 1990; Johnson et al. 2008; Niyogi et al. 1998; Winter and Königer 1989).

Niyogi et al. (1998) found that treating A. thaliana wild type plants with 2 mM DTT was sufficient to inhibit nonphotochemical quenching to a level similar to that of the npq1 mutant. Bailey and Walker (1992) treated the leaves used in their study with 40 mM DTT, but they passively allowed their leaves to uptake the inhibitor through the vascular tissue for 10 min rather than floating their leaves in it for 1.5 h and forcing the chemical through the tissue via vacuum infiltration, as we did in the present study, which likely resulted in lower effective concentrations of DTT accumulating in the leaves that they used. However, since we found that 40 mM DTT more effectively inhibited nonphotochemical quenching than 30 mM DTT in E. crassipes, the species with the leaves for which it was the most difficult to force the inhibitor through via vacuum infiltration, we elected to use the higher concentration in our study (Figure 2). Preliminary work by Kwon (2011) also suggested that concentrations of DTT lower than 30
mM using the same leaf disc treatment procedure did not fully suppress nonphotochemical quenching in wild type *A. thaliana* to the level of the *npq1* mutant. Perhaps DTT becomes more disruptive to other cellular processes at the higher concentrations experienced in our experiments compared to those to which leaves were subjected in previous studies.

DTT is also known to inhibit the photoprotective enzyme ascorbate peroxidase (often abbreviated as APO or APX), which is involved in scavenging oxygen radicals (Chen and Asada 1992; Neubauer 1993). When a plant’s capacity for photochemistry becomes overwhelmed, excess electrons flowing through the electron transport chain at photosystem I are often transferred to oxygen (O$_2$) instead of ferredoxin, forming superoxide radicals (O$_2^-$). These potentially damaging superoxide radicals can be scavenged and safely converted to water through a mechanisms known as the Mehler-ascorbate peroxidase (MP) reaction or the water-water cycle (Asada 1991; Mehler 1951). In this cycle, the enzyme superoxide dismutase (SOD) converts superoxide radicals to hydrogen peroxide (H$_2$O$_2$) and O$_2$, which subsequently react with ascorbate (vitamin C) via ascorbate peroxidase to form water and an oxidized ascorbate molecule which is converted back to ascorbate through a series of enzymatic reactions (Asada 1991).

Neubauer (1993) found that treating intact *Lactuca sativa* (lettuce) and *Spinacia oleracea* (spinach) chloroplasts with concentrations of DTT over 0.7 mM had a detrimental effect on ascorbate peroxidase activity and that treatment with 10 mM DTT decreased ascorbate peroxidase activity by 80%. Even though Neubauer (1993) used a more direct method of treating isolated chloroplasts with DTT rather than whole leaf tissue, as we did, the three-fold higher DTT concentration used in our study may have severely suppressed the scavenging of superoxide radicals formed under excess light exposure due to the inhibition of ascorbate peroxidase. The inhibition of this photoprotective mechanism may mean that the DTT-induced
depressions in yield recovery we observed cannot be exclusively attributed to the inactivation of violaxanthin de-epoxidase.

It was interesting that the *A. thaliana chup1* mutant recovered to a similar level as its wild type and *npq1* counterparts when its capacity for nonphotochemical quenching was inhibited, considering that the mutant is deficient in chloroplast movement (Table 7). Despite inhibition with DTT, the mutant recovered 43% of its original photochemical efficiency, even slightly more than the other two *A. thaliana* genotypes under DTT inhibition (Figure 8). The fact that this mutant was able to recover any of its pre-stress photosynthetic yield after exposure to high light stress when inhibited with DTT suggests that chloroplast movement and zeaxanthin-dependent nonphotochemical quenching are not the only two relevant processes involved in photoprotection. While zeaxanthin-independent nonphotochemical quenching is likely responsible for some of the *chup1* mutant’s ability to recover from high light exposure under DTT treatment, the mutant did not demonstrate exceptionally high levels of DTT-insensitive nonphotochemical quenching (Figure 4). Perhaps the *chup1* mutant has a heightened antioxidant system which allows it to efficiently scavenge the extra reactive oxygen species generated under excess light exposure due to its inability to avoid excess light absorption. Golan *et al.* (2006) demonstrated that photoprotection mutants, albeit deficient in nonphotochemical quenching rather than chloroplast movement, were almost completely able to avoid photooxidative damage via increased pools of the antioxidant compounds α-tocopherol (vitamin E) and ascorbate (vitamin C). The *chup1* mutant may also be able to compensate for its deficiencies in photoprotective traits with an enhanced ability to repair photooxidative damage. Plants that are less facile chloroplast movers, for example, have been observed to have greater capacities to repair the fragile D1 protein of photosystem II, compared to plants that utilize chloroplast movement to a greater extent (Park et
al. 1996). Since the degradation and repair of D1 proteins has been observed to occur within an hour of damage (Melis 1999), it is possible that the repair of damaged proteins is partially responsible for the recovery of photosynthetic yield that we observed in our stress treatments and that some of the variation that we observed in the resilience of plants to light stress may be due to differences in their abilities to repair damage.

**Greater Nonphotochemical Quenching Capacity Is Not Correlated with Enhanced Resilience to Light Stress**

Surprisingly, plants that displayed greater abilities to perform nonphotochemical quenching did not necessarily demonstrate an enhanced ability to recover from high light exposure compared to their counterparts that performed nonphotochemical quenching lesser degrees (Figure 9). The lack of an observed photoprotective advantage attributed to plants which presumably invested greater resources in their capacity for nonphotochemical quenching, such as larger xanthophyll pigment pools induced by light stress, suggests that plants may rely heavily on other photoprotective processes. Perhaps a stronger association between nonphotochemical quenching capacity and light stress resilience would be uncovered if a greater range of species were examined or if, as discussed earlier, the experimental plants were grown under more stressful light conditions, which would potentially extend the range of variation in nonphotochemical quenching capacity.

Intriguingly, we observed a negative, and much stronger, correlation between the capacities of plants to perform zeaxanthin-independent nonphotochemical quenching and recover from high light stress when treated with DTT (Figure 9). This relationship could be partially due to the fact that all three of the *A. thaliana* genotypes exhibited similar levels of zeaxanthin-
independent nonphotochemical quenching to the other species, but demonstrated severely attenuated abilities to recover from high light stress when treated with DTT (Figures 4 & 8). A. thaliana plants may be inherently more susceptible to potential non-target effects of DTT, perhaps due to a greater reliance on the Mehler-ascorbate peroxidase reaction to mitigate photodamage (Neubauer 1993).

**Nonphotochemical Quenching and Chloroplast Movement Capacities Are Uncorrelated**

Contrary to our original hypothesis, the ability of plants to perform nonphotochemical quenching and light-directed chloroplast relocation movements did not appear to be correlated (Figure 10). It would seem logical that species that are able to move their chloroplasts with great facility may not have a great need to mitigate photodamage through nonphotochemical quenching because they are able to physically avoid excess light absorption, while species that are not able to move their chloroplasts well may have a greater demand for thermal dissipation, but this trend was not observed (Figure 10).

Alternatively, it also seems logical that species that are well adapted to environments with frequent light stress exhibit greater capacities for both photoprotective processes. To evaluate this hypothesis, we compared the abilities of DTT-treated leaf tissue to recover from high light stress treatments with the abilities of CytB-treated leaf tissue to recover from identical stress treatments performed by Kwon (2011). Since our buffer-treated (control) leaf discs demonstrated a similar level of resilience to light stress as those of Kwon’s (2011) control leaf discs for most species and genotypes, we can confidently compare most of our data. The only discrepancy between our yield recovery data and Kwon’s data was with the A. thaliana chup1 mutant, which recovered an average of 72% of its photosynthetic capacity in our study, but only
26% in Kwon’s. This discrepancy may partially explain why *chup1* appears to be an outlier in relationships of NPQ$_{\text{max}}$ and DTT-inhibited light stress resilience with CytB-inhibited light stress resilience (Figure 11C & D). Overall, plants that showed greater resilience to light stress in the absence of chloroplast movement also tended to better able to recover from high light when their capacities for zeaxanthin-dependent nonphotochemical quenching were inhibited (Figure 11D), there did not appear to strong relationships between the two photoprotective processes (Figures 10 & 11).

The lack of an association or apparent trade-off between these two photoprotective mechanisms suggests that processes other than chloroplast movement and nonphotochemical quenching may play major roles in photoprotection. Photosynthetic capacity may also be a major factor determining the ability of plants to recover from high light stress. Plants with large capacities for photosynthesis may also be inherently better able to recover from high light exposure because they are able to use greater amounts of light for photochemistry and consequently do not have to cope with as much excess light as their counterparts with photosynthetic capacities that become saturated at lower light levels. We observed that *T. officinale* had the greatest capacity for photochemistry, measured as qP, after exposure to 12 min of high light (Figure 6), as well as one of the strongest recoveries of photosynthetic capacity after high light stress (Figure 8). However, qP was similar between the other plants studied and did not appear to be closely related to the ability of plants to recover from light stress. Perhaps examining the capacity of plants to quench absorbed energy through photochemistry after longer periods of high light exposure would reveal more dramatic differences in photosynthetic capacities that would explain difference in the recovery of plants from light stress that are not attributed to nonphotochemical quenching or chloroplast movement.
Conclusions and Future Directions

Overall, we found that different species are able to perform nonphotochemical quenching to various degrees and that plants that exhibited greater capacities for nonphotochemical quenching did not necessarily demonstrate enhanced resilience to high light stress. Contrary to our hypothesis, we did not observe a relationship between the abilities of the plants we studied to perform nonphotochemical quenching and light-induced chloroplast rearrangements.

While this may suggest that nonphotochemical quenching and chloroplast movement are not the only important photoprotective processes on which plants rely during high light stress, it is important to consider that the plants we used in our experiments were grown under relatively low light conditions and were thus not acclimated to high light. Perhaps if we grew our plants under higher light conditions, the potential differences in the photoprotective traits of our different species and *A. thaliana* genotypes would become more pronounced. Characterizing nonphotochemical quenching in a broader range of species, such as the selection fully studied by Königer and Bollinger (2012) and Kwon (2011), may also allow us to more confidently assess the potential relationship between nonphotochemical quenching and chloroplast movement.

The fact that our negative control, the *A. thaliana npq1* mutant, exhibited a severely attenuated ability to recover from high light stress when treated with DTT, despite that it is already deficient in violaxanthin de-epoxidase, also suggests that the inhibitor we used in this study has relevant side effects. It seems likely that the concentration of DTT used in our experiments disrupts ascorbate peroxidase activity and perhaps performing stress treatments with a weaker concentration of DTT may allow us to more accurately estimate the photoprotective importance of nonphotochemical quenching across different species.
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