Transactions of the Kansas
Academy of Science

Vol. 113, no. 3-4
p. 177-190 (2010)

Changes in chlorophyll content and antioxidant capacity during dark to light transitions in etiolated seedlings: Comparisons of species and units of enzyme activity

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Etiolated seedlings from *Triticum aestivum* L. (wheat), *Zea mays* L. (maize), *Lens culinaris* Medik. (lentil), *Pisum sativum* L. (pea), and *Phaseolus vulgaris* L. (bean) were studied during a four day period following transition into light. De-etiolation was expected to induce oxidative stress in seedlings, which would necessitate increased antioxidant enzymes. *Triticum* and *Phaseolus* seedlings were relatively quick to increase chlorophyll (chl) levels in light, whereas dark-germinated *Zea*, *Lens*, and *Pisum* seedlings had lower chlorophyll contents compared to light-germinated seedlings after four days of light exposure. The chl *a*/chl *b* ratio began significantly higher in light-germinated seedlings compared to dark-germinated seedlings. Chl *a*/chl *b* ratios were not significantly different between light- and dark-germinated treatments within two days of light exposure in *Triticum* and *Phaseolus*, but were still different in *Zea*, *Lens*, and *Pisum* after four days of light exposure. Leaf ascorbate peroxidase (APX) activities were higher in etiolated seedlings compared to light-germinated seedlings. By 3-4 days of light exposure, APX activities in dark-germinated seedlings had decreased to levels similar to light-germinated seedlings in most species. Leaf catalase activities were higher in light-germinated seedlings compared to dark-germinated seedlings. Following 4 days of light exposure, catalase activities in light-germinated seedlings remained higher than in dark-germinated seedlings. Thus, these species all respond similarly to oxidative stress during de-etiolation, but to different extents and at different rates. Moreover, different expressions of enzyme activities can take on different meanings, depending on the cellular location of the enzyme and the physiological changes occurring in that location.

Key Words: Ascorbate peroxidase, catalase, de-etiolation, greening, oxidative stress

Introduction

Early development in plants is accompanied by many structural, physiological, and biochemical changes. Formation of chloroplasts is known to be a time of increased oxidative stress in plants (Gillham and Dodge 1985). Light exposure in etiolated seedlings facilitates chloroplast development, but different components of the photosynthetic machinery develop at different rates. The pigment and electron transport system develop earlier than carbon reduction enzymes (Gillham and Dodge 1985), leading to a great potential for the formation of reactive oxygen species. $NADP^+$ will commonly be limiting as an electron acceptor while etioplasts transition into chloroplasts, and electrons are often passed to O_2 (Mattagajasingh and Kar 1989). For example, production of hydrogen peroxide (H_2O_2) has been shown to accompany development of chloroplasts (Laurenzi et al. 1999). Thus, increasing development in the light might be expected to result in increasing need to detoxify reactive oxygen species.

The abundance of oxygen and the high reductive potential of chloroplasts make them a rich source of reactive oxygen species (Allen 1995), often generated at PSI in thylakoids.

Any conditions that lead to excess reduction of PSI can lead to reduction of O_2 to superoxide anion ($^{\bullet}O_2^-$), including high light (Demmig-Adams and Adams 1992), cool temperatures (Posmyk et al. 2005), or closure of stomata (Allen 1995), e.g., due to drought or salinity.

Plants employ a diverse system of antioxidants to limit damage caused by reactive oxygen species, including the enzymes catalase, superoxide dismutase (SOD), and ascorbate peroxidase (APX), and nonenzymatic compounds like ascorbate and glutathione (Slesak et al. 2008; Kavitha, Venkataraman, and Parida 2008). When electrons are passed to O_2 at PSI, $^{\bullet}O_2^-$ is formed (i.e., the Mehler reaction), which is then dismuted to H_2O_2 by SOD. H_2O_2 is reduced to H_2O by thylakoid or stroma APX, with electrons from ascorbate, as part of the "water-water" cycle in chloroplasts (Asada 1999).

Reactive oxygen species also occur elsewhere in the cell, where different methods are involved in detoxification. Catalase is another enzyme to reduce H_2O_2 to H_2O. High activities of catalase are normally expressed in tissues of aerobic organisms (Harris 1990), but the location of catalase in peroxisomes limits its ability to counter oxidative damage in chloroplasts (Allen and Ort 2001). Nonetheless, catalase has been shown to be important for fighting oxidative stress in tobacco (Willekens et al. 1997).

Whereas H_2O_2 is itself damaging (e.g., it can inhibit enzymes of the Calvin Cycle; Charles and Halliwell 1981), H_2O_2 can also react with transition metals (e.g., iron) to form more damaging hydroxyl radicals ($^{\bullet}OH$) via the Haber-Weiss reaction (Asada 1992). $^{\bullet}OH$ is among the most toxic of all reactive oxygen species (Pfister-Sieber and Brandle 1994), and is thought to be responsible for the majority of oxidative damage in plants (Allen 1995). There are no scavengers of $^{\bullet}OH$ in plants (Apel and Hirt 2004). Therefore, it is beneficial for plants (and all other aerobic organisms) to detoxify H_2O_2 effectively in tissues before additional damage can occur (Asada 1992). Accordingly, catalase is one of the most abundant enzymes in plant and animal cells (Harris 1990), and catalase becomes important during times that facilitate formation of reactive oxygen species. Catalase activities have increased in plant tissue exposed to numerous varieties of biotic or abiotic stress (e.g., Nayyar and Gupta 2006; Wang et al. 2009). Ascorbate peroxidase (APX) plays a similar role in chloroplasts, where it is able to scavenge H_2O_2 and reduce it to H_2O (Nakano and Asada 1981). Thus, APX activities have been found to increase in plants under similar environmental conditions (e.g., Maricle, Cobos, and Campbell 2007; Wang et al. 2009).

There have been few reported measures of changes in antioxidant enzymes during the transition from etiolated to green states in seedlings (e.g., Appleman 1952; Feierabend and Beevers 1972; Tomomatsu and Asahi 1978; Gillham and Dodge 1985; Mattagajasingh and Kar 1989; Acevedo, Skadsen, and Scandalios 1996). Moreover, none of these studies involved a comparison between species. Different species could respond differently to oxidative stress during greening, so it is potentially beneficial to compare leaf-level processes between species. Additionally, previous studies expressed enzyme activities with a number of different units (e.g., activity per unit protein, per unit chlorophyll, or per unit mass). During the greening process, a number of changes occur, including rapid changes in chlorophyll and protein synthesis (Gillham and Dodge 1985). Therefore, expression of these enzyme activities can depend greatly on the units used. Each of these units has different meaning, and it is not known how these various expressions of activity compare with each other.

In the present study, etiolated seedlings from two grass and three legume species were studied during a four day period following transition to light. Leaf chlorophyll

concentrations were measured daily, as well as activities of total leaf ascorbate peroxidases (EC 1.11.1.11) and leaf catalase (EC 1.11.1.6). Enzyme activities were measured on a leaf mass basis, on a protein basis, and on a chlorophyll basis. Transition to light was expected to induce formation of reactive oxygen species in seedlings, which would necessitate increased antioxidant enzymes.

Abbreviations: ANOVA = analysis of variance; APX = ascorbate peroxidase; chl = chlorophyll; H_2O_2 = hydrogen peroxide; $NADP^+$ = Nicotinamide adenine dinucleotide phosphate, oxidized form; $^{\bullet}O_2^-$ = superoxide anion; $^{\bullet}OH$ = hydroxyl radicals; PSI = photosystem I; SOD = superoxide dismutase

Materials and Methods

Plant Material and Growing Conditions

Seeds of *Triticum aestivum* L. (wheat), *Zea mays* L. (maize), *Lens culinaris* Medik. (lentil), *Pisum sativum* L. (pea), and *Phaseolus vulgaris* L. (bean) were purchased commercially and germinated in dark or light conditions. Dark conditions were achieved by germinating seeds in a closed cabinet; light conditions were achieved by germinating seeds in a south-facing window (PPFD peaked around 400 µmol quanta m^{-2} s^{-1} at midday). Daytime temperatures were near 27°C in the light and near 25°C in the closed cabinet. Five pots of seeds (n=5) were started for each species in each treatment. Each pot included at least five seeds, which were sampled daily following transition to light.

Seedlings were allowed to germinate under dark or light conditions for 7 to 13 days, depending on species, to reach a size favorable for leaf measurements. At this point (day 0), all plants were transferred to lighted conditions. Transition from dark to light occurred 10 days after planting in *Triticum*, at 7 days in *Zea*, 13 days in *Lens*, and 11 days in *Pisum* and *Phaseolus*. Leaves of one plant from each pot (n=5) were sampled on days 0 to 4 (5 repeated samplings). Chlorophyll content, total ascorbate peroxidase (APX), and catalase activities were measured daily in leaf tissues during the transition from etiolated to green tissue.

Leaf Extractions

All leaves (not including petioles, but including the rachis and petiolules in compound leaves) from one seedling were harvested each day following the dark-to-light transition. Cold extraction buffer was added at 10 mL g^{-1}, which contained 50 mM Tris-HCl (pH 7.0), 5 mM $MgCl_2$, 2 mM cysteine hydrochloride, and 2% w/v PVP-40 (modified from Wang et al. 2009). Leaves were ground thoroughly with a chilled mortar and pestle.

40 µL of grindate was drawn up from the mortar and mixed with 960 µL of 100% ethanol. Chlorophyll contents (µg mL^{-1}) were determined spectrophotometrically after Wintermans and de Mots (1965) as:

(1)
chl *a* (µg mL^{-1}) = (13.70 x A_{665}) – (5.76 x A_{649})

(2)
chl *b* (µg mL^{-1}) = (25.80 x A_{649}) – (7.60 x A_{665})

(3)
total chl (µg mL^{-1}) = (6.10 x A_{665}) + (20.04 x A_{649})

(4)
Leaf chl (mg chl/g leaf) = chl concentration (µg /mL) x (1 mg /1000 µg)(0.04 mL/0.004 g)

The remaining grindate was centrifuged at 15,000 x g at 4°C for 5 minutes. The supernatant was assayed for ascorbate peroxidase (APX) and catalase activity colorimetrically at 25°C. Soluble protein concentrations were determined after Bradford (1976), with bovine serum albumin as the protein standard.

Leaf APX assays were performed spectrophometrically following a procedure of Nakano and Asada (1981). 10 µL aliquots

of the extract supernatant were added to 980 µL of a reaction mixture containing 50 mM K-phosphate buffer (pH 7.0), 0.5 mM ascorbate, and 0.1 mM EDTA. Background rates of ascorbate oxidation were calculated in the presence of enzyme extract but in the absence of H_2O_2. The reaction was initiated with the addition of 10 µL of 10 mM H_2O_2 (final H_2O_2 concentration of 0.1 mM in a total volume of 1.000 mL). Enzyme activity was determined as a decrease in ascorbate, measured as a decrease in absorbance at 290 nm (molar extinction coefficient of ascorbate at 290 nm is 2.8 mM^{-1} cm^{-1}). APX activities were corrected for background rates of ascorbate oxidation, then standardized to fresh leaf mass, to mg protein, and to chlorophyll content.

Leaf catalase activity was measured spectrophotometrically after Lee, Kim, and Lee (2001). 20 µL aliquots of extract supernatant were added to 980 µL of a reaction mixture containing 50 mM K-phosphate buffer (pH 7.0) and 10 mM H_2O_2 (total volume of 1.000 mL). Enzyme activity was determined as a decrease in H_2O_2, measured as a decrease in absorbance at 240 nm (molar extinction coefficient of H_2O_2 at 240 nm is 39.4 mM^{-1} cm^{-1}). Prior to addition of extract, the background rate of H_2O_2 reduction was measured. The assay was initiated by the addition of 20 µL of leaf extract. Catalase activities were corrected for background rates of H_2O_2 reduction, then standardized to fresh leaf mass, to mg protein, and to chlorophyll content.

Statistical Analyses

All data were analyzed using repeated measures analysis of variance (ANOVA). Species and treatments were fixed effects, and individual pots were the repeated effect (StatView 5; 1998 SAS Institute, Inc.; Cary, NC, USA). Post-hoc comparisons were performed using a Bonferroni correction. All analyses were performed at $\alpha = 0.05$.

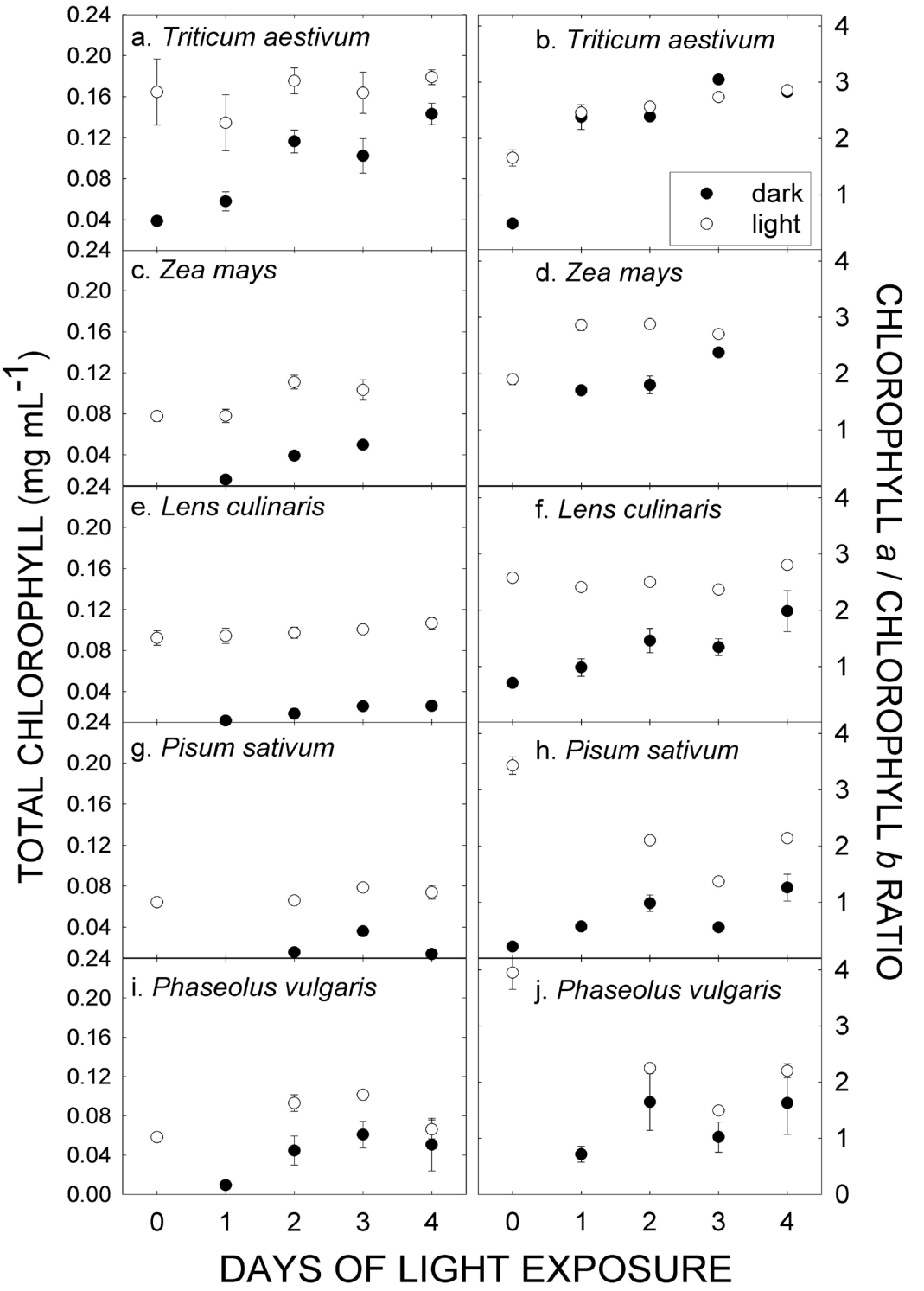
Results

Leaf Chlorophyll Content

All dark-germinated species were etiolated when they were transferred into light. Extracted leaf total chlorophyll ranged from 0.00 to 0.04 mg mL^{-1} in etiolated leaves across species at day 0, but increased to 0.01 to 0.14 mg mL^{-1} across species after four days of light exposure (Fig. 1). *Triticum* had a significantly higher chlorophyll content compared to all other species, followed by *Zea* and *Lens*, in turn significantly higher than *Phaseolus* and *Pisum* (Fig. 1; ANOVA, $p \leq 0.0003$). Chlorophyll contents were significantly lower in dark-germinated seedlings compared to light-germinated seedlings (Fig. 1; ANOVA, $p < 0.0001$). Chlorophyll contents did not significantly increase in *Pisum* during four days of light exposure (Fig. 1*g*). Significant increases in chlorophyll content were observed in all other species (ANOVA, $p \leq 0.0005$). After four days of light exposure, chlorophyll content in dark-germinated seedlings of *Phaseolus* was not different from light-germinated seedlings (Fig. 1*i*).

All light-germinated seedlings had a significantly higher chl *a*/chl *b* ratio compared to dark-germinated seedlings (Fig 1; ANOVA, $p < 0.0001$). This difference was greatest at day 0 but decreased in all species during the experiment (ANOVA, $p < 0.0001$). Moreover, there were no differences in chl *a*/chl *b* between dark- or light-germinated treatments after one day of light exposure in *Triticum* (Fig. 1*b*) or after two days of light exposure in *Phaseolus* (Fig. 1*j*). *Triticum* had a significantly higher chl *a*/chl *b* ratio compared to *Zea* and *Lens*, in turn significantly greater than *Phaseolus* and *Pisum* (Fig. 1; ANOVA, $p \leq 0.0020$).

Figure 1 (Right). Leaf chlorophyll contents and chl *a*/chl *b* ratios from dark-germinated (filled circles) and light-germinated (open circles) seedlings during four days of light exposure. Points are means ± SE of five replicates; in some cases the error bars are smaller than the points.


a. Triticum aestivum
b. Triticum aestivum
c. Zea mays
d. Zea mays
e. Lens culinaris
f. Lens culinaris
g. Pisum sativum
h. Pisum sativum
i. Phaseolus vulgaris
j. Phaseolus vulgaris
dark
light
TOTAL CHLOROPHYLL (mg mL-1)
CHLOROPHYLL a / CHLOROPHYLL b RATIO
DAYS OF LIGHT EXPOSURE
0.24
0.20
0.16
0.12
0.08
0.04
0.00
4
3
2
1
0
0 1 2 3 4

Leaf Enzyme Activities

On a fresh mass basis, leaf APX activities ranged from 1.9 to 10.3 µmol g^{-1} min^{-1} in etiolated seedlings at day 0, but increased to 2.1 to 11.4 µmol g^{-1} min^{-1} across species after four days of light exposure (Fig. 2). Leaf catalase activities were 185 to 1356 µmol g^{-1} min^{-1} in dark-germinated seedlings at day 0, and increased to 224 to 2620 µmol g^{-1} min^{-1} after four days in the light (Fig. 3). Neither leaf APX activities nor catalase activities in light-germinated seedlings had an effect of time (Figs. 2-3). Leaf APX activities were significantly higher in dark-germinated seedlings compared to light-germinated seedlings (Fig 2; ANOVA, $p=0.0066$). By contrast, leaf catalase activities were significantly higher in light-germinated seedlings compared to dark-germinated seedlings (Fig 3; ANOVA, $p<0.0001$). Similar patterns of enzyme activity were observed between species. *Triticum* had consistently high leaf APX and catalase activities, which were significantly higher than in *Phaseolus*, *Lens*, and *Pisum* (Figs. 2-3; ANOVA, $p\leq0.0014$). Leaf APX activities in *Zea* were among the highest compared to the other species, but leaf catalase activities in *Zea* were among the lowest compared to other species (Figs. 2-3).

Similar patterns existed between enzyme activities expressed on a fresh mass basis and on a per-protein basis. On a per-protein basis, leaf APX activities ranged from 0.5 to 9.1 µmol mg^{-1} protein min^{-1} in dark-germinated seedlings and from 0.4 to 1.4 µmol mg^{-1} protein min^{-1} in light-germinated seedlings (Fig. 2). Leaf catalase activities ranged from 57 to 923 µmol mg^{-1} protein min^{-1} in etiolated seedlings and from 140 to 1381 µmol mg^{-1} protein min^{-1} in light-germinated seedlings (Fig. 3), with a slight increase through time (Fig 3; ANOVA, $p<0.0001$). Leaf catalase activities were significantly higher in light-germinated seedlings compared to dark-germinated seedlings (Fig 3; ANOVA, $p<0.0001$). By contrast, there was no difference in leaf APX activity between dark- and light-germinated treatments when expressed on a per-protein basis (Fig. 2; ANOVA, $p=0.324$). In terms of species comparisons, similar patterns existed between enzyme activities based on leaf mass and activities per unit protein (Figs. 2-3).

Leaf APX and catalase activities at day 0 appeared higher in etiolated seedlings compared to light-germinated seedlings when compared on a per-chlorophyll basis (Figs. 2-3). Leaf APX activities at day 0 ranged from 26.4 to 98.7 µmol mg^{-1} chl min^{-1} across etiolated plants, compared to 3.2 to 8.0 µmol mg^{-1} chl min^{-1} in light-germinated plants. Leaf catalase activities at day 0 were 909 to 2984 µmol mg^{-1} chl min^{-1} across plants that were dark germinated, compared to 328 to 3898 µmol mg^{-1} chl min^{-1} in light-germinated plants. When expressed on a per-chlorophyll basis, however, there were no significant differences in APX or catalase activities between species, treatments, or their interactions (ANOVA, $p\geq0.523$) owing to a great deal of variation between dark-germinated individuals. By days 3 and 4, light- and dark-germinated treatments all appeared uniform in terms of both leaf APX and catalase activities in dark- and light-germinated seedlings (Figs. 2-3).

Discussion

Activities of antioxidant enzymes were followed to assess oxidative stress during de-etiolation in seedlings. In the present study, all etiolated seedlings responded to light with the synthesis of chlorophyll. Whereas etiolated *Triticum* and *Phaseolus* seedlings were relatively quick to increase chlorophyll levels, dark-germinated *Zea*, *Lens*, and *Pisum* seedlings still had much lower chlorophyll contents compared to light-germinated seedlings after four days of light exposure. Leaf chlorophyll contents in *Pisum* were lower than those reported by Mackerness, Jordan, and Thomas (1999) for pea buds or by Gillham and Dodge (1985) for pea leaves. This is most likely due to the inclusion of rachis and petiolule tissue in the present study, as the

compound leaves of *Pisum* and *Lens* showed the slowest accumulation of chlorophyll in dark-germinated leaves (Fig. 1).

The chl *a*/chl *b* ratio began significantly higher in light-germinated seedlings compared to dark-germinated seedlings. In most cases, chl *a*/chl *b* ratios ranged from 2.0 to 3.0 for species in this study, similar to values for *Cajanus cajan* L. presented by Kumutha et al. (2009), but slightly lower than values for *Zea mays* presented by Holá et al. (2007). This difference is potentially due to brighter light in the greenhouses used by Holá et al. (2007), compared to the window used in the present study. Growth in low light increases the need for large light-harvesting complexes, which are typically rich in chlorophyll *b* (Anderson and Anderson 1988). Thus, moderate light in a window would decrease the chl *a*/chl *b* ratio (Fig. 1) compared to leaves under brighter light in a greenhouse (Holá et al. 2007). Furthermore, chl *a*/chl *b* ratios would be lowest in dark-germinated plants, evident at day 0 (Fig. 1). Increasing chl *a*/chl *b* ratios during light exposure across species indicate a decreased need for extensive light-harvesting complexes, which can be disassociated in brighter light (Anderson and Anderson 1988). Chl *a*/chl *b* ratios were not significantly different between light- and dark-germinated treatments within two days of light exposure in *Triticum* and *Phaseolus*, but were still different in *Zea*, *Lens*, and *Pisum* after four days of light exposure. Thus, there were no evident taxonomic or morphological patterns in relation to the chl *a*/chl *b* ratio in developing leaves.

Exposure to light will also increase the synthesis of ascorbate (Asada 1992), which is needed for many lines of defense against oxidative stress (Alla and Hassan 2006), including mediation of the reduction of H_2O_2 by ascorbate peroxidase (APX). In the present study, leaf APX activities were higher in etiolated seedlings compared to light-germinated seedlings. This is similar to previously-published results in rice seedlings, where APX was prevalent in etiolated plants, and viewed to be active in reducing H_2O_2 formed by mitochondria prior to chloroplast development (Komatsu, Muhammad, and Rakwal 1999). Work on etiolated wheat seedlings by Mattagajasingh and Kar (1989) showed a spike of leaf APX activity 2 hr after illumination, but a decrease thereafter. Although the present study did not investigate hourly resolution, APX activities in *Triticum*, *Zea*, and *Phaseolus* appeared to increase during the first day of illumination, then decreased to previous levels. This is potentially consistent with the previously-published results in rice.

Leaf APX activities were variable between species, and were generally lower than previously-published values for salt marsh grasses (Maricle, Cobos, and Campbell 2007) or *Zea mays* (Alla and Hassan 2006), and were similar to values in *Avicennia marina* (Forsk.) Vierh. (Kavitha, Venkataraman, and Parida 2008), *Lycopersicum pennellii* Mill. (Shalata et al. 2001), *Solanum tuberosum* L. (Benavides et al. 2000), and *Medicago sativa* L. (Wang et al. 2009), but were higher than values presented for *Phragmites communis* Trin. (Chen et al. 2007). In the present study, leaf APX activities were not different between etiolated and light-germinated seedlings when compared on a per-chlorophyll basis. This is particularly important, as chloroplasts will be a major source of reactive oxygen species in plants (Allen 1995).

Catalase, which is in peroxisomes, also helps counter oxidative damage by reducing H_2O_2 to H_2O. Despite its damaging nature, H_2O_2 is a common product in plant metabolism. Highly reduced intermediates in chloroplasts (e.g., PSI; Allen 1995) or in mitochondria (e.g., complexes I-III; Turrens 2003) can ultimately lead to formation of H_2O_2 if electrons are passed to oxygen. As a result, H_2O_2 reduction is needed throughout the cell, not just in chloroplasts as mediated by APX. Catalase is a ubiquitous enzyme across aerobic life

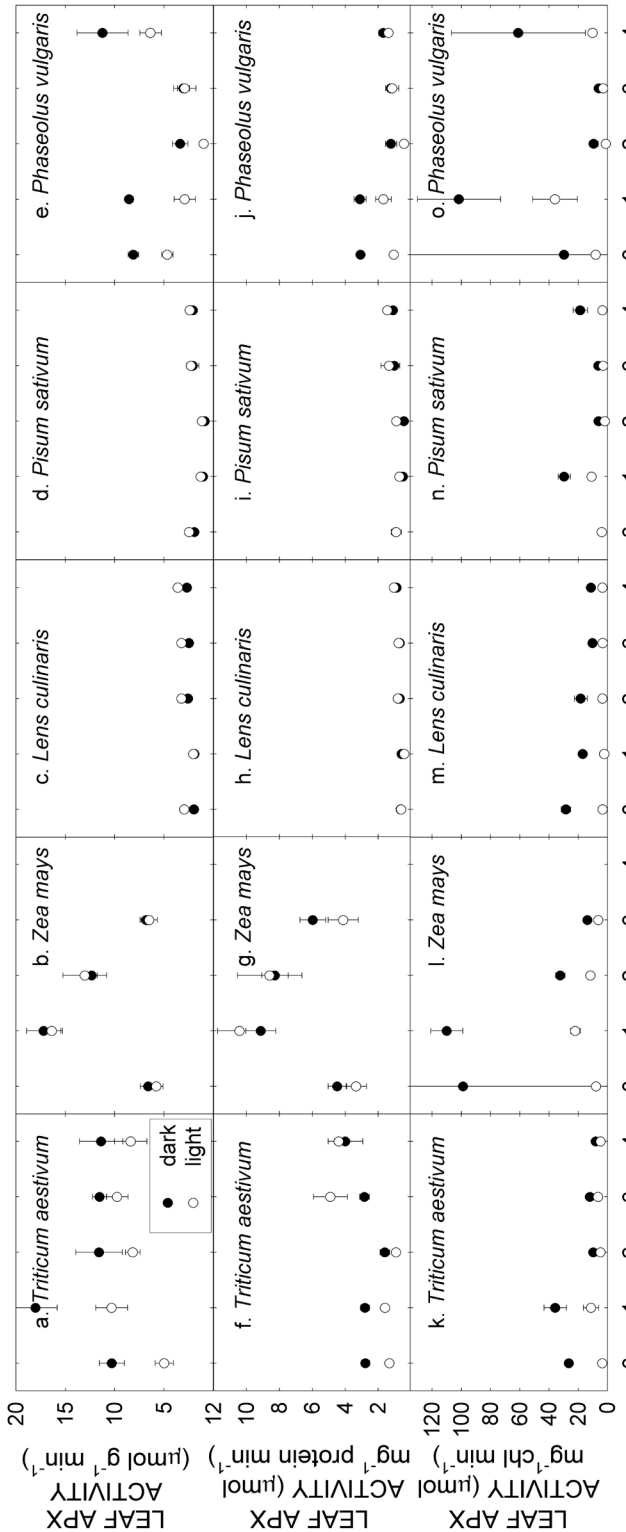


Figure 2. Leaf total ascorbate peroxidase (APX) activities from dark-germinated (filled circles) and light-germinated (open circles) seedlings during four days of light exposure. APX activities are expressed on a fresh mass basis (panels *a-e*), on a mg protein basis (panels *f-j*), and on a mg chlorophyll basis (panels *k-o*). Points are means ± SE of five replicates; in some cases the error bars are smaller than the points. The near-zero (slightly negative) chlorophyll content of dark-germinated *Pisum* at day 0 resulted in an activity below zero.

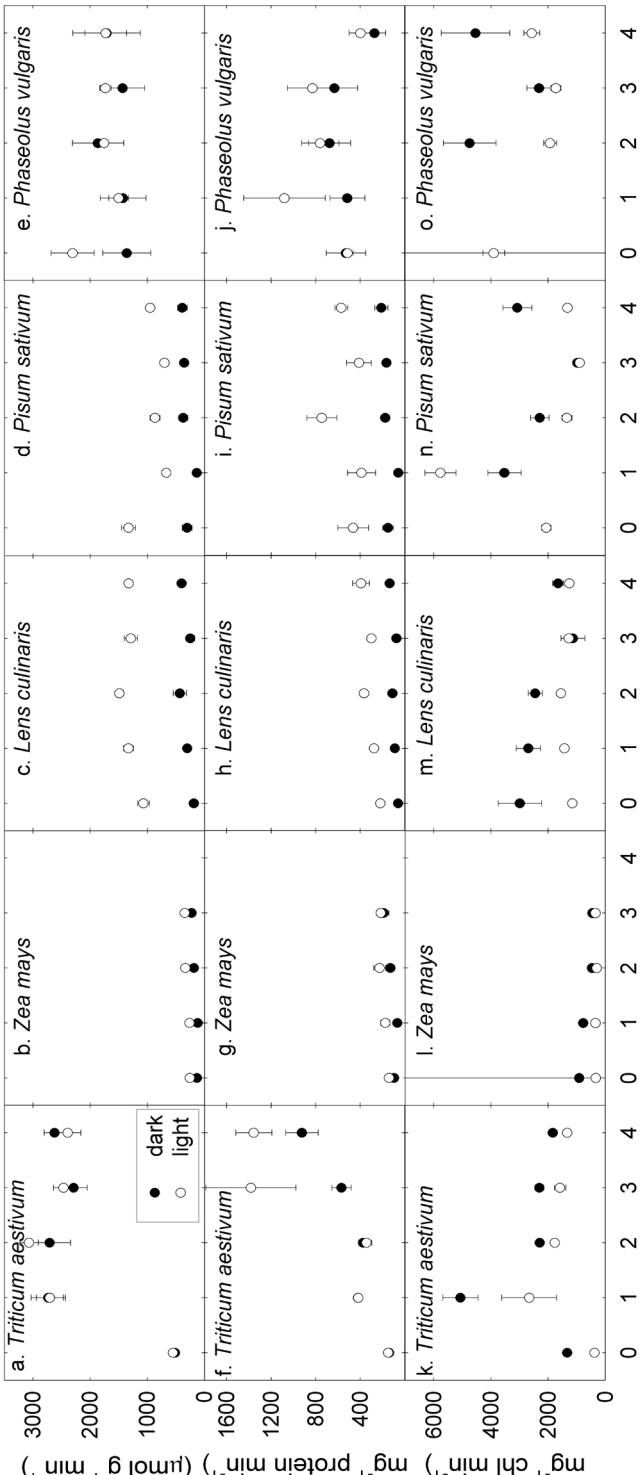


Figure 3. Leaf catalase activities from dark-germinated (filled circles) and light-germinated (open circles) seedlings during four days of light exposure. Catalase activities are expressed on a fresh mass basis (panels *a-e*), on a mg protein basis (panels *f-j*), and on a mg chlorophyll basis (panels *k-o*). Points are means ± SE of five replicates; in some cases the error bars are smaller than the points. The near-zero (slightly negative) chlorophyll content of dark-germinated *Pisum* at day 0 resulted in an activity below zero.

(Stryer 1995), where it occurs in copious amounts and helps counter oxidative stress in aerobic organisms (Harris 1990). In the present study, leaf catalase activities were highest in *Triticum* and *Phaseolus*, without significant differences between treatments (Fig. 3). *Triticum* was the only species that increased catalase activities after exposure to light. This is similar to previous studies on *Triticum* (Feierabend and Beevers 1972; Mattagajasingh and Kar 1989), where catalase activities increased in etiolated seedlings after exposure to light, but unlike a previous study on *Phaseolus* (Tomomatsu and Asahi 1978) where catalase activities also increased in etiolated seedlings after exposure to light. In the present study, leaf catalase activities were slightly lower in *Zea*, *Lens*, and *Pisum*, where light-germinated leaves had significantly higher catalase activities compared to dark-germinated leaves. When expressed on a per-chlorophyll basis, leaf catalase activities appeared to begin higher in dark-germinated seedlings compared to light-germinated seedlings (nonsignificant difference). This was simply due to lower amounts of chlorophyll in dark-germinated seedlings, as catalase activities were significantly higher in light-germinated seedlings when expressed on a leaf-mass or mg-protein basis. Although neither catalase nor APX activities were different between species or treatments when compared on a per-chlorophyll basis, catalase activities are most likely not as meaningful as APX activities when considering chlorophyll development, as chloroplasts will be a major source of reactive oxygen species in plants (Apel and Hirt 2004). Nonetheless, catalase activities can be meaningful as they indicate H_2O_2 scavenging ability in the cell away from chloroplasts. Moreover, cytosolic isoforms of APX commonly increase activity during environmental stress, as H_2O_2 leaks into the cytosol from mitochondria and peroxisomes (Hernandez et al. 2000). Thus, an ability to detoxify H_2O_2 is a prerequisite for coping with many types of biotic or abiotic stress. Indeed, a large ability to scavenge H_2O_2 is regarded as an important feature of salt-tolerant plants (Verma and Mishra 2005).

Both catalase and APX catalyze the reduction of H_2O_2 to H_2O. Whereas catalase is limited to peroxisomes, there are eight isoforms of APX throughout the plant cell, including forms in plastids, cytosol, and peroxisomes (Hong et al. 2007). There is a definite advantage to having this redundancy in plant cells. APX has high affinity for H_2O_2 and an ability to detoxify H_2O_2 at low concentrations. In contrast, catalase has a lower affinity for H_2O_2 but a much higher reaction rate (Hong et al. 2007). Thus, a combination of the two is an effective defense against H_2O_2, which might be important as a general mechanism for dealing with environmental stress. For example, the ability to tolerate salinity might be related to the ability to increase antioxidant capacity to combat the associated oxidative stress (Hernandez et al. 2000; Maricle, Cobos, and Campbell 2007; Sekmen, Türkan, and Takio 2007; Arbona et al. 2008). The results of this study indicate de-etiolation of seedlings under low light induces modest oxidative stress. But this is offset by an increase in catalase activity and decrease in APX activity, the combination of which makes dark-germinated seedlings equivalent to light-germinated seedlings in H_2O_2 scavenging ability.

Antioxidant activities are expressed with a number of different units (e.g., activity per unit protein, per unit chlorophyll, or per unit mass). Each of these units is used widely, and it is unknown how they compare to each other. Due to the changing nature of proteins and chlorophyll in a developing seedling, expression of enzyme activities can depend greatly on the units used.

In the present study, there were no significant differences detected in APX or catalase activities expressed on a per chlorophyll basis. There was a great deal of variation in

chlorophyll content between dark-germinated individuals. This resulted in a high degree of variation in all enzyme activities, and no detectable significant differences, despite cases where there were obvious differences between treatments. When working with plants during a period of rapid chlorophyll synthesis, it appears best to avoid expression of enzyme activities on a per chlorophyll basis.

There were several differences between species and treatments when enzyme activities were compared on a per protein basis. Differences in APX activity were not detected between light- and dark-germinated treatments, but catalase activity was significantly greater in light-germinated seedlings compared to dark-germinated seedlings. Catalase activity was higher in light-germinated seeds, most likely because the antioxidant system takes longer than four days to reach maximal levels (Feierabend and Beevers 1972; Tomomatsu and Asahi 1978).

An interesting difference was noted when enzyme activities were compared on a fresh mass basis. Leaf APX activities were significantly higher in dark-germinated seedlings compared to light-germinated seedlings. By contrast, leaf catalase activity was significantly higher in light-germinated seedlings compared to dark-germinated seedlings. Higher APX activity in dark-germinated seedlings might represent a burst of oxidative stress with the onset of chloroplast development during greening (Gillham and Dodge 1985). However, catalase activities were significantly greater in light-germinated seedlings compared to dark-germinated seedlings. This is due to greater development of antioxidant systems in light-germinated seedlings (Tomomatsu and Asahi 1978).

When expressing enzyme activities, it is important to consider the cytological location of the enzyme and the relevant physiological changes that are occurring in relation to the enzyme. In the present example, leaf APX activities were greater in dark-germinated seedlings during greening compared to light-germinated seedlings, most likely because the greening of chloroplasts is accompanied by a burst of oxidative stress (Mattagajasingh and Kar 1989). The large difference in APX activity during greening potentially reflects chloroplast isoforms of APX. By contrast, catalase occurs exclusively in peroxisomes, which are insulated from oxidative bursts during chloroplast maturation. Instead, catalase was slower to develop in dark-germinated seedlings, as it takes longer than four days to adjust to lighted conditions and the oxidative stress it might create in the cell (Feierabend and Beevers 1972; Tomomatsu and Asahi 1978). Therefore, the appropriate units to use for enzyme activities can involve any of the above. But activities per unit chlorophyll should be avoided during times of rapid chlorophyll synthesis, and activities per unit mass or per unit protein can take on different meanings depending on the cellular location and the expected physiological changes occurring in that location.

Conclusion

Seedlings in the present study had similar responses to light exposure, but at different rates and to different extents. All species had lower chlorophyll contents and lower catalase activities in etiolated seedlings compared to green seedlings. *Triticum* and *Phaseolus* appeared to be the quickest to develop chlorophyll and increase catalase activity following light exposure. In contrast, leaf APX activities were higher in etiolated seedlings compared to green seedlings, and decreased following light exposure. In most species, leaf APX activities were equal between light- and dark-germinated seedlings following two or three days of light exposure, with *Zea*, *Lens*, and *Pisum* adjusting most quickly.

Acknowledgments

This study was partially funded by the Department of Biological Sciences and the Fort Hays State University Graduate School Small Research Grant. I thank Sam R. Zwenger and Aaron M. Pfeifer for help with lab measurements.

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