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# Effect of High Light intensity on Photoinhibition, Oxyradicals and Artemisinin Content in Artemisia annua L.

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# Effect of high light intensity on photoinhibition, oxyradicals and artemisinin content in *Artemisia annua* L. ♠

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## Abstract

*Artemisia annua L.* produces a compound called artemisinin that is a potent anti-malarial compound. However concentration of artemisinin within the plant is typically low (less than 0.8% of dry mass) and currently supply of the drug by the plant does not meet world demand. This investigation was carried out to determine whether high intensity light treatment would increase production of artemisinin in leaves of *A. annua*. Photoinhibition (14%) was induced in leaves of *A. annua* when they were subjected to 6 h of high-intensity light [2,000 μmol(photon) $m^{-2}$ $s^{-1}$]. Maximum photochemical efficiency of PSII showed a recovery of up to 95% within 24 h of light induced inhibition. During the light treatment, photochemical efficiency of PSII in leaves of the high-intensity light-treated plants was 38% lower than for those from leaves of plants subjected to a low-intensity-light treatment of 100 μmol(photon) $m^{-2}$ $s^{-1}$. Non-photochemical quenching of excess excitation energy was 2.7 times higher for leaves treated with high-intensity light than for those irradiated with low-intensity-light. Elevation in oxidative stress in irradiated leaves increased presence of reactive oxygen species (ROS) including singlet oxygen, superoxide anions, and hydrogen peroxide. Importantly, the concentration of artemisinin in leaves was two-fold higher for leaves treated with high-intensity light, as compared to those treated with low-intensity light. These results indicate that *A. annua* responds to high irradiance through nonphotochemical dissipation of light energy yet is subject to photoinhibitory loss of photosynthetic capacity. It can be concluded that *A. annua* is capable of rapid recovery from photoinhibition caused by high light intensity. High light intensity also induces oxidative stress characterized by increased concentration of ROS which enhances the content of artemisinin. Such a light treatment may be useful for the purpose of increasing artemisinin content in *A. annua* prior to harvest.

*Additional key words*: chlorophyll fluorescence; environmental stress; irradiation.

## Introduction

*Artemisia annua* L. is an aromatic annual herb that is native of temperate Asia. It produces a compound called artemisinin, which is an endoperoxide-bridged sesquiterpene lactone synthesized in glandular trichomes (Pandey *et al.* 1999). Artemisinin is currently one of the most effective drugs for treatment of malaria and is recommended by the World Health Organization (WHO) in the form of artemisinin-based combination therapy (ACT). Artemisinin has also been shown to have activity against viral diseases including hepatitis B and C (Efferth *et al.* 2008) and nonmalarial parasitic infections including schistosomiasis (Krishna *et al.* 2008) and leishmaniasis (Sen *et al.* 2010). Because the concentration of artemisinin in *A. annua* tissues is extremely low [0.01–0.8% of dry mass (DM); van Agtmael *et al.* 1999] the availability of an adequate supply of the compound to meet world demand has traditionally been a problem (Abdin *et al.* 2003).

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*Abbreviations*: ACT – artemisinin-based combination therapy; APX – ascorbate peroxidase; FM – fresh mass; $F_m$ – maximal fluorescence yield of the dark-adapted state; $F_m'$ – maximal fluorescence yield of the light-adapted state; $F_s$ – steady-state fluorescence yield; $F_v$ – variable fluorescence; $F_v/F_m$ – maximal quantum yield of PSII photochemistry; HPLC – high pressure liquid chromatography; NPQ – nonphotochemical quenching of PSII; ROS – reactive oxygen species; $q_P$ – photochemical quenching coefficient; SD – standard deviation; UV-B – ultraviolet-B; UV-C– ultraviolet-C; $\Phi_{PSII}$ – quantum efficiency of PSII; WHO – World Health Organization.

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Recently, a protocol for efficient semisynthetic production of artemisinic acid, a precursor of artemisinin, and for the conversion of artemisinic acid to artemisinin using singlet oxygen has been reported (Paddon *et al.* 2013). This methodology of artemisinin synthesis has the potential to provide a stable, inexpensive source of artemisinin to meet the world-demand for ACT. However, methods to increase artemisinin content in plants prior to extraction are of still of great interest since there is evidence that other compounds found in *A. annua* work synergistically with artemisinin for enhanced efficacy against malaria (Ferreira *et al.* 2010) and cancer (Ivanescu and Corciova 2014). Further, it would be more economically feasible to produce artemisinin through plant extraction methods in certain locations where malaria is prevalent (Brisibe and Chuckwurah 2014).

There is great variability in the artemisinin content from different accessions of *A. annua* (Delabays *et al.* 2001). Increased leaf artemisinin content has been achieved using traditional plant breeding techniques (Delabays *et al.* 2001) and specific genetic-loci affecting the yield of artemisinin have been identified (Graham *et al.* 2010). Artemisinin content is dependent on the developmental stage of plants (Ferreira *et al.* 1995) and is variable for harvested plant tissue depending on postharvest drying methods used (Ferreira and Luthria 2010). Soil nitrogen and potassium availability affects biomass and artemisinin production for *A. annua* in the field (Ferreira 2007, Davies *et al.* 2009) and preharvest treatment of plants with plant growth regulators such as $GA_3$ and triconatrol (Aftab *et al.* 2010a, Banyai *et al.* 2011), mechanical wounding of leaves (Liu *et al.* 2010), application of the fungicide, chitosan, (Lei *et al.* 2011) and water deficit (Charles *et al.* 1993, Marchese *et al.* 2010) lead to an increase in artemisinin production for plants harvested.

Treatment of *A. annua* with reactive oxygen species (ROS) or environmental conditions that lead to the production of ROS including lead and soil salinity (Qureshi *et al.* 2005) and dimethyl sulfoxide addition (Mannan *et al.* 2010) also tend to increase artemisinin production in leaves. Enhanced production of ROS that correlated with higher artemisinin levels were documented in response to environmental stresses such as chilling (Feng *et al.* 2009), exposure to boron (Aftab *et al.* 2010b) and cadmium (Li *et al.* 2012) and application of the antibiotic fosmidomycin (*Zeng et al.* 2011). Salicylic acid treatment also induced artemisinin production, and alleviated salinity-induced oxidative stress and loss of photosynthetic capacity for *A. annua* (Pu *et al.* 2009, Aftab 2011).

The objective of this study was to determine whether short-term exposure to high intensity light could be used to induce increase artemisinin production for leaves of *A. annua.* Wang *et al.* (2008) determined that although *A. annua* can grow in lower intensity light (15% full sun), growth at high intensity light (up to 100% full sunlight) leads to increased photosynthesis, growth and artemisinin production. Rai *et al.* (2011) found that treatments of UV-B (ultraviolet-B) and UV-C (ultraviolet-C) radiation prior to harvest enhance artemisinin and flavonoid content for the plant. The effect of a high-intensity visible-light pretreatment on artemisinin production has not been investigated. Because light that exceeds photosynthetic capacity of plants is known to be damaging to their photosynthetic machinery (*e.g.*, Kok 1956) and most plants are able to avoid light-induced damage through nonphotochemical quenching (NPQ) energy dissipation pathways (*e.g.*, Müller *et al.* 2001, Logan 2005) we investigated the capacity that *A. annua* has to dissipate excess light energy. Given the potential for enhanced ROS production in *A. annua* under photoinhibitory light conditions (Asada and Takahashi 1987, Asada 2006), we hypothesized that exposure of such conditions prior to harvest might enhance ROS-mediated artemisinin production in *A. annua* plants.

## Materials and methods

**Plant material**: Seeds of an ornamental strain of *A. annua* (*Horizon Herbs*, Oregon, USA) were sown in flats in a mixture of soil (*Sunshine Professional, Sun Gro Horticulture*, Bellevue, Washington, USA) and sand (1:1) and germinated in a chamber (*E-30B*, *Percival Scientific*, Iowa, USA) at 24°C. At four weeks, after the first true leaves were formed, plants were transplanted to 15 cm pots in the same medium and were grown in a greenhouse under a mix of natural and supplemental metal halide lamps to achieve 500 μmol(photon) $m^{-2}$ $s^{-1}$ of PAR. Plants were fertilized weekly and watered as needed. When plants were at least 90 but not more than 100 days old, a subset of plants was randomly selected and subjected to an irradiance treatment ranging between 100 and 2,000 μmol (photon) $m^{-2}$ $s^{-1}$ PAR for 6 h. Light was provided by 1000 W metal halide lamps (*Aurora Innovations*, Eugene, Oregon, USA) and the intensity of the light was controlled using neutral density filters. Light intensity was monitored with a quantum sensor (*LI-250, LI-Cor Inc.*, Lincoln, Nebraska, USA) and temperature throughout the treatment remained between 24 – 28 °C. Prior to and after the light treatment, *A. annua* plants were used for physiological measurements as described below.

**Chlorophyll fluorescence** was measured using a pulse-modulated fluorometer (*Fluorescence Monitoring System-1, Hansatech*, England, UK). Maximum dark-adapted photochemical efficiency of PSII ($F_v/F_m$; Genty *et al.* 1989) was measured after a 30 min dark-adapt period. Steady-state fluorescence yield ($F_s$) was monitored under light-adapted conditions, and maximal fluorescence yield of the light-adapted state ($F_m'$) was measured during a saturating pulse of white light. From these values photochemical efficiency of PSII [$\Phi_{PSII} = (F_m' - F_s)/F_m'$] the photochemical and nonphotochemical

quenching coefficients of fluorescence were calculated according to Genty *et al.* (1989) at 1 min, 15 min, 30 min, 1 h, and hourly intervals until 6 h during the light treatment.

**Superoxide ($O_2^{\bullet -}$)**: Superoxide production was measured according to the method of Able *et al.* (1998) as modified by Lei *et al.* (2011). Leaves of *A. annua* [500 mg fresh mass (FM)] were homogenized with 1.0 ml of 50 mM Tris–HCl buffer (pH 7.5) and centrifuged at 12,000 × *g* for 15 min at 4°C. The reaction mixture, in a final volume of 3.0 ml, contained 50 mM Tris–HCl (pH 7.5), 0.5 mM XTT (*Sigma-Aldrich*, St. Louis, Missouri, USA) and 100 µl of supernatant. The reduction of XTT was determined by measuring the absorbance at 470 nm in a spectrophotometer (*UV-2401 PC, Shimadzu*, Japan). The quantity of $O_2^{\bullet -}$ was determined using the molar extinction coefficient (ε) $2.16 \times 10^4$ $M^{-1}$ $cm^{-1}$ (Lei *et al.* 2011).

**Hydrogen peroxide ($H_2O_2$)**: The concentration of $H_2O_2$ in the *A. annua* leaf tissue was determined according to the method of Mukherjee and Choudhuri (1983) as modified by Pu *et al.* (2009). Leaves of *A. annua* leaves (500 mg FM) were homogenized with 5 ml precooled acetone and the homogenate was centrifuged at 12,000×g for 5 min. A volume of 1 ml of the supernatant was mixed with 0.1 ml of 5% $TiOSO_4$ (*Sigma-Aldrich*, St. Louis, Missouri, USA) and 0.2 ml 19% ammonia. After a precipitate was formed, the reaction mixture was centrifuged at 12,000 × *g* for 5 min. The resulting pellet was dissolved in 3 ml 2M $H_2SO_4$ and the absorbance was determined at 415 nm using a spectrophotometer (*UV-2401 PC, Shimadzu*, Japan). The $H_2O_2$ concentration (µM) was determined using a standard curve of $H_2O_2$ ranging from 0 to 10 µM.

**Singlet oxygen ($^1O_2$)**: Singlet oxygen was quantified spectrophotometrically by percentage bleaching at 440 nm ($A_{440}$), of the $^1O_2$ scavenger, N,N-dimethyl-p-nitrosoaniline using the methods of Feng *et al.* (2009) and Guo *et al*. (2010) with modifications. *A. annua* leaf tissue of 450 mg (FM) was put into 10 ml of assay mixture containing 45 mM PBS buffer (pH 7.1), 10 mM histidine and 50 µM N,N-dimethyl-p-nitrosoaniline (*Sigma-Aldrich*, St. Louis, Missouri, USA). The assay mixture was incubated at 30ºC under a 400 W metal halide lamp (*Aurora Innovations*, Eugene, Oregon, USA) at 320 µmol(photon) $m^{-2}$ $s^{-1}$ in order to facilitate assay bleaching. Absorbance at 440 nm was determined spectrophotometrically (*UV-2401 PC, Shimadzu*, Japan) at intervals of 0.5 h for a 2.5 h duration of the bleaching assay.

**Artemisinin concentration**: Artemisinin was extracted and quantified as per Han *et al.* (2006). Treated and control leaves were collected and dried for 24 h in an oven at 50°C. Dried plant material was ground to fine powder and 100 mg powder was extracted with 40 mL petroleum ether for 12 h. The mixture was sonicated for 2 min and filtered. Filtrates were evaporated to dryness, dissolved in 10 mL methanol and centrifuged at 12,000 × *g* for 5 min. Supernatant was collected and used as artemisinin extraction solution. One mL solution was mixed with 4 mL of 0.2% (w/v) NaOH and incubated in a 50°C water bath for 30 min. After cooling to room temperature with tap water, 5 mL of 0.05 mol/L acetic acid was added to the solution and mixed thoroughly. The mixture was filtered and then used for high pressure liquid chromatography (HPLC) assay. HPLC was performed under the following conditions: 3.9 × 150 mm $C_{18}$ reverse phase column, mobile phase 0.01 mol $L^{-1}$ sodium phosphate buffer (pH 7.0):methanol (50:50), flow rate 1 mL $min^{-1}$, and wavelength 260 nm. The injection volume was 10 µL. The retention time of artemisinin was approximately 3.7 min. The calibration curve was obtained using an artemisinin standard (*Sigma-Aldrich*, St. Louis, Missouri, USA).

**Statistical analysis**: All experiments were repeated with plants grown at different times of approximately the same age (90–100 days old). Oxidative stress assays were replicated eight times and artemisinin concentrations and chlorophyll fluorescence evaluations were replicated six times. *Student*'s *t*-tests were employed to determine statistically significant differences between treatment and control plants.

## Results

**Chlorophyll fluorescence**: Maximum quantum efficiency of PSII ($F_v/F_m$) decreased 14% after 6 h treatment with 2,000 µmol (photon) $m^{-2}$ $s^{-1}$ high light as compared to values measured prior to the treatment or for plants that were treated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ light (Fig. 1). Leaves treated with 500 or 1,000 µmol(photon) $m^{-2}$ $s^{-1}$ for 6 h had $F_v/F_m$ values similar to plants treated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ light (data not shown). After a 24-h low-light recovery period, photoinhibited plants recovered to 95% of their initial $F_v/F_m$ value. Upon exposure to 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ high light, $\Phi_{PSII}$ levels increased two times in value across the first 15 min of exposure, after which values stabilized throughout the remainder of the light treatment (Fig. 2). Over the course of the 6 h treatment, average NPQ values for plants treated with 2000 µmol(photon) $m^{-2}$ $s^{-1}$ light, were up to 20 times higher than those for plants treated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ light (Fig. 2). Plants treated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ light maintained a higher level of $\Phi_{PSII}$

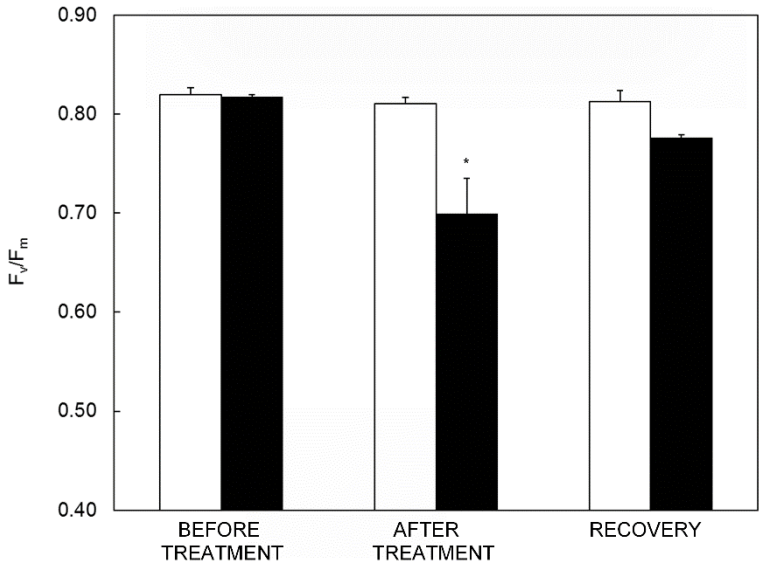


Fig. 1. Response of maximum photochemical efficiency of photosystem II ($F_v/F_m$) for *Artemisia annua* leaves before, and after, a 6-h exposure to 100 µmol(photon) $m^{-2}$ $s^{-1}$ (*open bars*) or 2000 µmol(photon) $m^{-2}$ $s^{-1}$ (*closed bars*). * – significant difference ($p$<0.01) between plants exposed to different light treatments. Bars represent means ± SD, $n$ = 6.

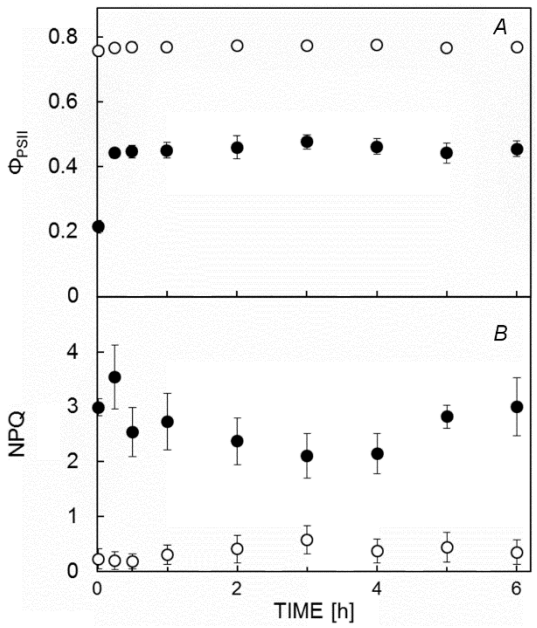


Fig. 2. Response of photochemical efficiency of photosystem II ($\Phi_{PSII}$, *A*) and of nonphotochemical quenching of fluorescence (NPQ, *B*) to time of exposure for *Artemisia annua* leaves exposed to 100 µmol(photon) $m^{-2}$ $s^{-1}$ (○) or 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ (●) for up to 6 h. Each time point represents means of measurements from 6 plants ± SD (some error bars are smaller than symbols). All differences for plants receiving different light exposures are statistically significant ($p$≤0.001). The first measurement was taken at one minute.

and a lower level of NPQ than plants treated with high intensity light throughout the 6h light treatment. Plants treated with 500 or 1,000 µmol(photon) $m^{-2}$ $s^{-1}$ light had $\Phi_{PSII}$ and NPQ values that were similar to those for plants treated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ light (data not shown). Photochemical quenching ($q_P$) values were up to 20% higher in leaves treated with 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ light, as compared to leaves treated with 100, 500 or 1,000 µmol(photon) $m^{-2}$ $s^{-1}$ light (data not shown).

**Oxidative stress**: To investigate the mechanism by which the 6-h irradiance treatment affected artemisinin production, concentrations of the reactive oxygen species (ROS) superoxide anion ($O_2^{\bullet -}$) hydrogen peroxide ($H_2O_2$), and singlet oxygen, ($^1O_2$) were assessed. Results of the $O_2^{\bullet -}$ assay indicated a 85% increase in $O_2^{\bullet -}$ for plants treated with 6 h of ,2000 µmol(photon) $m^{-2}$ $s^{-1}$ light, as opposed to 100 µmol(photon) $m^{-2}$ $s^{-1}$ (Fig. 3). The $H_2O_2$ concentration for leaves irradiated with 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ light increased by 35% after 6 h compared to those irradiated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ light (Fig. 3).

Production of $^1O_2$ in leaves after light exposure was monitored in a bleaching assay. The production of $^1O_2$ in leaf tissue leads to bleaching of a reaction media at 440 nm. The reaction medium is monitored for 2.5 h after completion of the light-treatment. Plants irradiated with low and high-intensity light exhibited a postillumination burst of $^1O_2$ (Fig. 4). The relative size of the burst of $^1O_2$ measured for plants irradiated with 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ was two times that from plants irradiated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ light (Fig. 4).

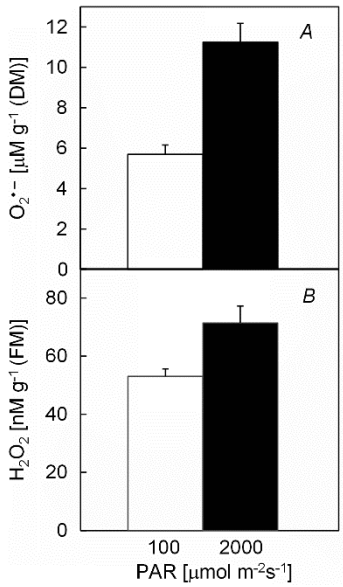


Fig. 3. Superoxide ($O_2^{\bullet -}$), *A*, and hydrogen peroxide ($H_2O_2$) production, *B*, for *Artemisia annua* leaves exposed to 100 µmol(photon) $m^{-2}$ $s^{-1}$ (*open bars*) or 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ (*closed bars*) for 6 h. Differences for plants receiving different light exposures are statistically significant ($p \leq 0.001$). Bars represent means ± SD, $n = 8$.

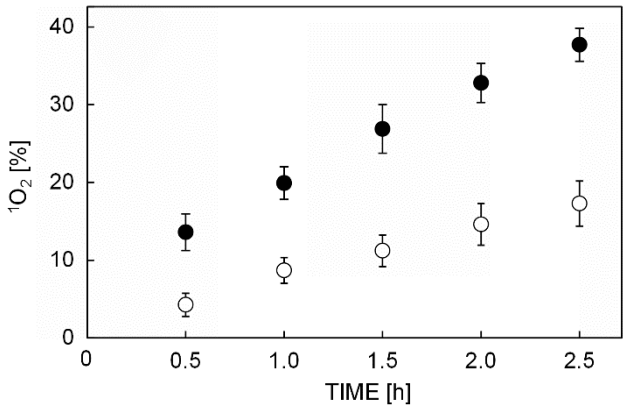


Fig. 4. Singlet oxygen burst from a 2.5 h bleaching assay that correlates with loss of $A_{440\ nm}$. $^1O_2$ emitted by *Artemisia annua* leaves exposed to 100 µmol(photon) $m^{-2}$ $s^{-1}$ (○) or 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ (●) for 6 h prior to bleaching assay. Relative strength of burst [%] = [$A_{440nm}$ of blank – $A_{440nm}$ of sample)/$A_{440nm}$ blank] × 100. Bars represent means ± SD, $n = 8$. Differences between treatments for each time point are statistically significant ($p<0.001$).

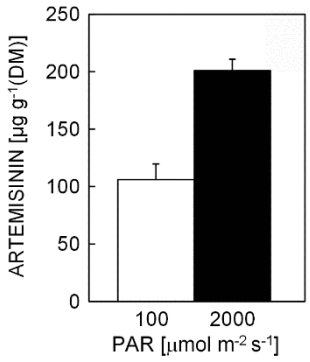


Fig. 5. Artemisinin concentration for *Artemisia annua* leaves exposed to 100 µmol(photon) $m^{-2}$ $s^{-1}$ (*open bars*) or 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ (*closed bars*) for 6 h. Bars represent means of measurements from 6 plants ± SD. Difference is statistically significant at $p \leq 0.01$.

**Artemisinin concentration**, as determined by assay with HPLC, was nearly two fold higher for plants irradiated with 2,000 µmol(photon) $m^{-2}$ $s^{-1}$ for 6 h, as compared to plants irradiated with 100 µmol(photon) $m^{-2}$ $s^{-1}$ (Fig. 5) for the same duration. Plants irradiated with lower-intensity irradiation had artemisinin concentration similar to that which was measured prior to the irradiation period (data not shown).

## Discussion

*Artemisia annua*, exhibited a small but measurable amount of photoinhibition resulting from a high irradiation treatment of 6 h, as indicated by decreased dark-adapted maximal photochemical efficiency of PSII (Kyle 1987). Overnight recovery was adequate for protein synthesis required to fully repair photosynthetic reaction centers (Ohad *et al.* 1993). Decreased photochemical efficiency of PSII in *A. annua* during the high intensity light exposure indicates that rapidly reversible energy dissipation reactions operated to increase nonphotochemical quenching of fluorescence (NPQ) during the light treatment (Genty *et al.* 1989, Horton *et al.*1996). *A. annua* is a plant that grows well in a range of light intensities and has increased photosynthetic rates at higher growth light-intensities up to full sun (Wang *et al.* 2008). This light-adaptability, coupled with the plants ability to effectively dissipate excess excitation energy even when grown at light intensities less than full sun appear to be adequate to limit photoinhibition during a 6-h high-light intensity treatment.

Good agricultural practices described for *A. annua* note that the plant naturally occurs and is best-grown in high-light intensity areas such as the edges of forests or in open fields (WHO 2006). Our results indicate that in cases where it is necessary to grow the plant in more shade-like areas due to space limitations or resource constraints, exposure to high-light prior to harvest could be used as a practical means for increasing postharvest artemisinin concentration in the leaves of *A. annua*. Since *A. annua* is able to recover from short term photoinhibition, it may be possible to expose mature plants to repeated high light intensity treatments with minimal photoinhibitory losses to plant productivity. However, because photoprotection mechanisms such as NPQ reduce photosynthetic capacity of plants, their initiation in the presence of high-light may lead to decreased plant yields that serve to offset the benefits of increased artemisinin production.

High irradiance produces ROS in chloroplasts at two prominent sites: oxygen can be reduced at PSI forming superoxide anions ($O_2^{\bullet -}$) and hydrogen peroxide ($H_2O_2$) and, if photoactivated chlorophyll cannot transfer its excitation energy at PSII, singlet oxygen ($^1O_2$) can be formed from long-lived triplet chlorophyll (Logan 2005, Asada 2006). Although plants have both non-enzymatic ROS-scavenging such as carotenoids and enzymatic scavenging mechanisms, such as superoxide dismutase (SOD) to catalyze $O_2^{\bullet -}$ conversion to $H_2O_2$ and further detoxification of $H_2O_2$ by ascorbate peroxidase (APX), oxidative stress occurs when production of ROS outpaces their dissipation (Asada 1999, Apel and Hirt 2004). Oxidative stress can lead to loss of plant productivity through lipid peroxidation, membrane leakage and eventually cellular death (Mishra and Choudhuri 1999, Triantaphylidès *et al.* 2008).

In this investigation, exposure of *A. annua* to high intensity light induced measurable production of $O_2^{\bullet -}$, $H_2O_2$, and $^1O_2$. At the same time, the light-treatment increased artemisinin production for the plants. Production of artemisinin in response to chilling stress via conversion of its immediate precursors was first suggested by Wallaart *et al.* (1999). A mechanism for abiotic-stress-induced artemisinin production was later proposed by Qureshi *et al.* (2005) who suggest that artemisinin precursors are converted into artemisinin through utilization of oxidative species produced in response to environmental stresses. Additional evidence for a catalytic mechanism for artemisinin production from its precursor, dihydroartemisinic acid, using the burst of ROS that occurs under abiotic stresses, has been given for *A. annua* exposed to boron (Aftab 2010b), and cadmium (Li *et al.* 2012) toxicity and through the application of DMSO (Mannan 2010).

In addition to production of artemisinin by conversion of precursors for the product, oxidative stress that is induced upon treatment of *A. annua* plants with salicylic acid or methyl jasmonate (Pu *et al.* 2009, Guo *et al.* 2010), fosmidomycin (Zeng *et al.* 2011) or UV radiation (UV-B or UV-C, Rai *et al.* 2011) has been shown to increase artemisinin production through a signaling mechanism that includes upregulation of artemisinin biosynthetic genes. Pu *et al.* (2011) gave evidence that $^1O_2$ production upon exposure of plants to salicylic acid increased artemisinin on a relatively short time-scale (measureable differences occurred 8 h after treatment) through both mechanisms: conversion of dihydroartemisinic acid to artemisinin and upregulation of genes encoding for artemisinin biosynthesis. Given the relatively short time scale or our exposure of *A. annua* to high-light, it seems likely that increased production of artemisinin observed in this study was induced by conversion of precursors into artemisinin through a ROS-mediated processes however induction of artemisinin *via* a signaling event mediated with or without ROS is also possible within this time range and cannot be ruled out without further research.

On a broader scope of increasing artemisinin production for developing countries, we have provided evidence that suggests artemisinin production can be enhanced when leaves are irradiated with full sunlight for a 6 h period. Continuous exposure of *A. annua* to high intensity light induced mechanisms for nonphotochemical quenching of excess excitation energy and oxidative stress for plants thereby triggering increased production of artemisinin over a relatively short time scale. Recovery of *A. annua* from high intensity light-induced photoinibition within 24 h indicates that repeated exposure of the plant to high light intensity may serve to further increase artemisinin content of leaves without loss of plant yields. However, because oxidative stress also decreases plant yields, it may be that the benefits of high intensity light diminish over repeated exposure.

Methods as simple as harvesting *A. annua* from the field on clear days with sunny skies may be useful for increasing artemisinin yield for farmers as well as small-scale growers. Unfortunately in many growth locations, such as those in which *A. annua* plants are completely shaded, exposure of plants to high intensity light may not be practical without artificial lighting. However in locations where at least partial or directional sunlight is available for at least some hours of the day, our results indicate that harvest after maximum high intensity light exposure should serve to increase yields of artemisinin for *A. annua* in the field.

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