Article

Pyrroloquinoline Quinone Treatment for Prevention of Mitochondrial Damage in Plants

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1. ABSTRACT

Mitochondrial decay inflicted by oxidative stress in brain cells is the primary cause of neurodegenerative disorders such as Alzheimer's and strokes. Oxidative stress is an imbalance of oxygen caused by disproportional amounts of pro-oxidants and antioxidants, which regulate the consumption of radical oxygen species (ROS). Overproduction of ROS disrupts cell structure of lipids, membranes, proteins, DNA, and also attacks mitochondria. The naturally slow rate of mitochondrial genesis is harmful to cells undergoing oxidative stress, as mitochondria cannot reproduce fast enough to replenish the necessary mitochondria count in the cell, leading to cell death. Pyrroloquinoline quinone (PQQ) is a vitamin that reduces ROS while accelerating mitochondrial growth, therefore making it a factor in resisting damage from oxidative stress. This experiment introduced antioxidant PQQ into Wisconsin Fast Plants undergoing oxidative stress. Plants were grown in varying concentrations of PQQ solution and exposed to oxidative stress once mature. The 0.1% PQQ solution led to the best plant survival, decaying at a slower rate. However, all plants watered with the vitamin did not grow as tall as the control. Therefore, PQQ intake at 0.1 percent concentration allows an extended lifespan for Wisconsin Fast Plants when exposed to oxidative stress, but the organism will be compromised in overall height.

2. INTRODUCTION

Mitochondrial decay inflicted by oxidative stress in brain cells is the primary cause of all neurodegenerative disorders such as Alzheimer's and strokes (Kobayashi et al., 2006). According to recent estimates, stroke is the second most common global cause of mortality and the third most common cause of death in more developed countries (Sarti, Rastenyte, Cepaitis, & Tuomilehto, 2000). Case-fatality of total strokes varies little between populations, mostly between 20–30% (Feigin, Lawes, Bennett, & Anderson). 11.84% of global deaths were caused by neurological diseases in 2015 (World Health, 2006). Alzheimer's, Parkinson's, strokes, and other neurodegenerative diseases are caused by several factors, but all damage primarily stems from oxidative stress in the brain (Kobayashi et al., 2006).

The naturally slow rate of mitochondrial genesis is harmful to cells undergoing oxidative stress, as mitochondria cannot reproduce fast enough to replenish its numbers, eventually leading to irreversible damage and sometimes death (Friederich, Hansell, & Palm, 2009; Halliwell, 1992; Sena & Chandel, 2012). The overall mitochondria count of an organism further declines as an organism ages; existing mitochondria grow weaker and lose function while the rate of production of new mitochondria is insufficient to replenish mitochondrial loss (Kobayashi et al., 2006). These weaker mitochondria are even more likely to be damaged by ROS (Kobayashi

et al., 2006).

Recent work has shown that oxidative stress can be chemically induced in order to provide working models for studying oxidative stress (Li et al., 2003). Hydrogen peroxide (H202), a reactive oxygen species (ROS), was put in cerebral vascular smooth muscle cells to determine if this increase in free-radicals is a cause of strokes and neurodegeneration. Following H2O2 treatment, muscle cells underwent apoptosis, stimulated by the death of mitochondria. Mitochondria count continually declined until ATP (adenosine triphosphate) production did not reach sufficient levels to support survival (Deavall, Martin, Horner, & Roberts, 2012; Li et al., 2003). Similar research by Barry Halliwell confirms H2O2 as a reactive oxygen species capable of inducing apoptosis through oxidative stress (Halliwell, 1992).

Pyrroloquinoline quinone (PQQ) is a vitamin that reduces ROS while accelerating mitochondrial growth, and therefore has the potential to be used to treat or prevent oxidative stress. A PQQ-rich diet should positively impact the health of an organism as it ages (Chowanadisai et al.). Recent work has tested the effects of PQQ in vivo, with rat diet serving as the independent variable (Ohwada et al.). The experimental group, which was fed a PQQ-supplemented diet, resulted in better memory and information retention than the control groups (Ohwada et al.). A similar experiment tested the in vitro effects of PQQ in cardiac rat muscles, which provided similar results (Kobayashi et al., 2006). Heart cells treated with PQQ supplements experienced less cell death than untreated cells when exposed to H2O2 (Kobayashi et al., 2006). An article published by BioMed Research International extensively explains the mechanism behind PQQ by explaining how the natural interaction between pro-oxidants and antioxidants naturally fights oxidative stress (Rahal et al., 2014). This article concludes that antioxidants reduce an overabundance of ROS. Both of these rat experiments support the hypothesis that a PQQ-concentrated diet could potentially reduce risk of oxidation damage. However, if an excess of antioxidants are present, too many ROS may be consumed, which can also lead to negative effects (Rahal et al., 2014).

Similar to mammals, plants also experience oxidative stress (Bartosz, 1997). Although plants do not have neural or glial cells, an overabundance of ROS can have negative effects. An increase in antioxidants such as PQQ may help prevent this damage, as it has the potential to consume excess ROS and strengthen existing mitochondria. Oxidative stress is more significant in plants than animals because unlike other organisms, plants produce their own amino acids rather than obtaining them from ingestion. Therefore, all amino acids must be produced internally through protein synthesis. If synthesis is slowed or stopped due to oxidative stress, the plant will rapidly decline in health until it can no longer function (Mühlenbock, Karpinska, & Karpinski, 2001).

Herein, Wisconsin Fast Plant seeds were grown under a 24-hour light source and watered via a watering wick system. The plants were grown in the same conditions with the same soil for three weeks until mature. The solution taken up by the watering wick served as the independent variable as each plant had a specific concentration of PQQ dissolved in its water. The control group had a solution of pure water while the experimental groups were watered with varying concentrations of PQQ solution. After three weeks of growth, the plants were exposed to identical hydrogen peroxide solutions to induce oxidative stress. Observations were made following one week of over-oxidation with continued normal watering regimens. In this work, the preventative effects of PQQ on susceptibility to ROS-induced damage are investigated. We hypothesize that high levels of PQQ will prevent oxidative stress damage to a greater degree than an organism that ingests lower PQQ levels.

3. MATERIALS

Water bottles purchased at Kroger (Gahanna, OH), an X-Acto Knife (Home Depot, Gahanna OH), 4-inch watering wicks (Home Depot, Gahanna, OH), a hammer, nails, potting mix (Home Depot, Gahanna, OH), and Wisconsin Fast Plant seeds (Carolina.com), 10 mg PQQ capsules (Jarrow Formulas), distilled water, a 24-hour Agrobrite grow light, balance, soap, 35% hydrogen peroxide (Momentum98, Columbus, OH).

4. METHODS

4.1 Preparation of Pots and Watering Wick System

Water bottles were emptied and cleaned with soap and water. Each bottle was horizontally cut in half directly under the label. A hole was created in the center of the lid with a hammer and nail. A watering

wick was woven through the hole in all bottles. The top half of each bottle (the half containing the lid) was turned upside-down and placed inside the corresponding bottom half to create a pot (Figure 1).

Figure 1. The watering pots and watering wick system. The colored labels indicate a specific solution (blue=0.5% PQQ solution, pink=0.25% PQQ solution).

Each pot was filled to the top with potting mix. Three seeds were placed in the center of each pot.

4.2 Preparation of PQQ-Concentrated Watering Solutions
Equation 1 was used to determine the mass of PQQ needed for each solution.

Equation 1
(% desired as a decimal) x (500 mL) = grams of PQQ

Solutions (500 mL) containing 0.01%, 0.1%, 0.25%, and 0.5% PQQ concentration were made. 50 mL of each solution was placed into the bottom portion of the appropriately labeled pot (n=10). Untreated water served as a control (n=10).
4.3 Plant Growth
All plants were placed under the growth light for three weeks. Watering solutions were replenished as necessary. Yellow blossoms formed after about two and a half weeks (Figure 2). Plant height was measured with a ruler in mm. Stem color was measured on a 0-5 color scale, shown in section 4.4.

Figure 2. After two and a half weeks, all watering solutions yielded flowering plants. This picture shows the flowers grown in 0.25% PQQ solution.

4.4 Chemically Induced Oxidative Stress
Hydrogen peroxide was diluted to 20% concentration using the formula below:
Equation 2
C1V1=C2V2
10 mL of diluted H2O2 solution was directly poured into the soil of each pot and observations were recorded after one and two days. Plant height was recorded with a ruler in mm. Stem color was also observed on a 0-5 color scale shown below by visual observation (Figure 3).

Figure 3. Color scale used for observation of stem color.

5. RESULTS
5.1 Plant Growth Prior to Over-Oxidation
After allowing the plants to grow for two and a half weeks until maturation, variation in plant height was recorded (Figure 4, Table 1). Plants grown in untreated watering solution (control group) showed the tallest average height amongst all groups. The 0.25% PQQ watering solution group yielded the second highest average height prior to hydrogen peroxide treatment, followed by the 0.01% PQQ solution, 0.1% PQQ solution, and the 0.5% PQQ solution,

respectively. The sample size for the 0.1% PQQ solution was reduced to 8 because 2 plants died prior to maturation. The sample size for the 0.5% PQQ watering solution was reduced to 6 because 4 plants died prior to maturation. Stem color was recorded based on the color scale in section 3.4 (Figure 3).

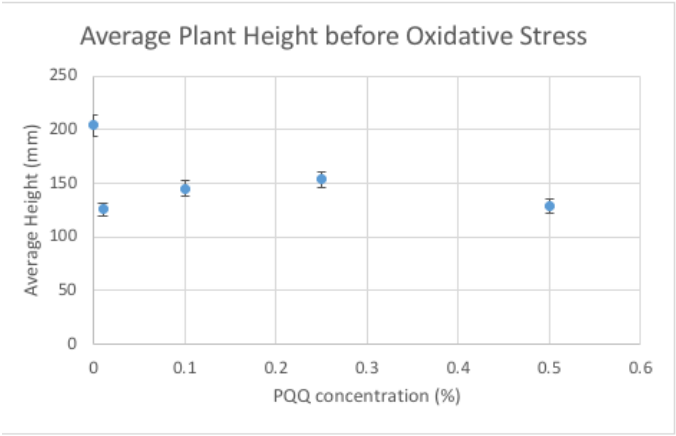

Figure 4. Average plant height before oxidative stress. The control group grew the tallest on average. The 0.01% PQQ solution and 0.5% PQQ solution plants were the shortest on average. (0.1% solution n=8, 0.5% n=6).

5.2 Effect of Hydrogen Peroxide on Growth and Survival

After chemically induced oxidative stress, the plant height was recorded (Figure 5, Table 1). 1 day after oxidative stress, the average plant height was measured for each experimental group and the control group. The 0.1% PQQ solution group was the tallest on average after treatment with concentrated hydrogen peroxide. After oxidative stress, visual observation concluded that all stems thinned and leaves became more fragile compared to initial growth. The color chart (Figure 3) was also used to observe stem color 1 day after hydrogen peroxide treatment and again 2 days after, shown in Table 1.

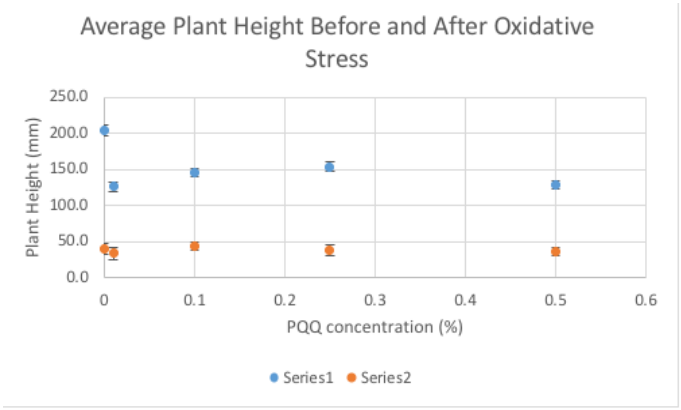

Figure 5. Average plant height after oxidative stress compared to initial growth. The 0.1% PQQ solution yielded the tallest plants on average after hydrogen peroxide treatment. The 0.01% PQQ solution plants were the shortest on average. (0.1% solution n=8, 0.5% solution n=6). Series 1=before oxidative stress, series 2= after oxidative stress.

Table 1. Average plant height before and after oxidative stress, and stem color changes. The control group had the tallest initial growth. After oxidative stress, the 0.1% PQQ concentration group was the tallest on average. The 0.1% group lived the longest, as it was the only group with a live plant 2 days after oxidative stress. Plant death was defined as stem color reaching 5.

PQQ Concentration (%)	Avg. height before (mm)	Avg. height after (mm)	Stem Color before	Stem color (Day 1)	Stem color (Day 2)
0	203.8	39.8	0	4	5
0.01	125.7	33.6	0	5	5
*0.1	145	43	0	2	3
0.25	153.5	37.7	0	5	5
**0.5	128.7	36.3	0	3	5

*Sample size=8
**Sample size=6

5.3 Comparative analysis before and after oxidative stress

The percent height differences were measured comparing average height before and after oxidative stress using equation 3.

Equation 3.

[(H0-Hf)/H0]x100%

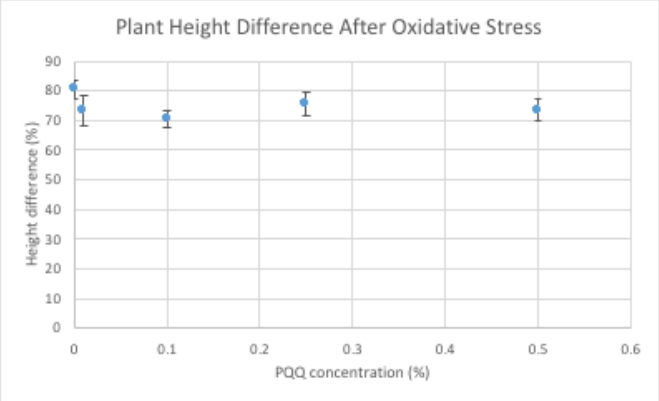

Figure 6. Average percent height difference after oxidative stress. The 0.1% PQQ solution group had the smallest percent height reduction on average. The control group had the highest percent height reduction on average. All of the PQQ experimental groups better maintained initial height compared to the control. (0.1% solution n=8, 0.5% solution n=6).

6. DISCUSSION

6.1 Initial Growth

All watering solutions yielded mature, flowering plants. However, the overall average height of the control group was the tallest, implying that PQQ solutions compromised plant growth (Figure 3). The mature plants of all watering solutions had the same initial stem color, suggesting that the vertically compromised plants were still healthy. Four of the ten plants watered with 0.5% PQQ solution died before reaching maturity, and two out of the ten plants watered with 0.1% PQQ solution also died upon initial growth. One of the 0.1% solution plants never grew, and the other 0.1% PQQ solution plant ran out of watering solution and was left untreated for two days, killing the plant. Therefore, the plant deaths in the 0.1% PQQ solution group may have been caused by operator error. Premature plant deaths in the 0.5% PQQ samples imply that PQQ has the potential to damage organisms when consumed in high concentrations. PQQ is an antioxidant, and a precise balance of pro-oxidants and antioxidants are required in the body for proper consumption of ROS (Rahal et al., 2014). Therefore, by adding excess antioxidants, that balance is--at least temporarily--disproportionate. The PQQ concentration of the lower percentage solutions did not disrupt the interplay of pro-oxidants and antioxidants enough to cause significant damage to the organism as was seen in previous work (Rahal et al., 2014).

6.2 Consequences of Oxidative Stress

One day after hydrogen peroxide treatment, the average height of the 0.1% PQQ solution group was the tallest, followed by the control group, 0.25% group, 0.5% group, and 0.01% group, respectively (Table 1). The percent height difference was also calculated (Figure 4). The 0.1% PQQ solution group best maintained its initial height after over-oxidation, recording a percent height difference of 108.5%. The 0.5% PQQ solution killed 4 plants prematurely, possibly because of a sizeable excess of antioxidants (Rahal et al., 2014). The intermediate PQQ solution concentration performed the best in maintaining initial height after hydrogen peroxide treatment, and therefore the mid-range PQQ concentration is concluded to be the best concentration out of the experimental groups in resisting oxidation damage. Further experimentation should be performed with more PQQ concentrations closer to 0.1% to determine more specific resistant levels.

All experimental groups and the control group experienced 100% plant death, meaning that oxidative stress killed all 50 plants. However, the rate of death varied greatly. Stem color was observed one day following H2O2 treatment (Table 1). Plant death was defined as stem coloration ranking a 5 on the color scale (Figure 3). The 0.1% PQQ solution group most closely maintained its original color, followed by 0.5%. Two days following oxidative stress, living plants only existed in the 0.1% solution experimental group (Table 1). The remaining plants died within the next 24 hours. This observation supports the conclusion that 0.1% PQQ solution best resisted oxidative stress damage.

A more dilute solution of hydrogen peroxide, such as 10-15%, may lead to more accurate results in future experimentation because the plants may have reacted more slowly to the hydrogen peroxide solution. An error occurred because of the inability to adequately replenish water supply in one of the 0.1% plants,

causing its premature death. The plants were left under the growth light, and over a long weekend, the solution evaporated, leaving the plant an estimated two days without a water source and killing it. Therefore, sample size for this group was reduced. Larger sample size for all plant groups would better verify the results of this experiment.

7. CONCLUSION

From this work, we conclude that the plants grown in 0.1% PQQ solution were able to survive the longest after oxidative stress, and they recorded the most minimal stem color change and percent height difference. However, all PQQ solution experimental groups did not grow as tall as the control, meaning the addition of PQQ compromised initial growth. However, these compromised plants still appeared healthy, as their stem coloration was the same as the control. The plants watered with PQQ solutions were able to withstand and resist damage from hydrogen peroxide slightly better than the control because they better maintained their initial height throughout experimentation. The data also concludes that mid-range PQQ solutions are more effective in oxidative stress-induced damage resistance. Four out of the ten plants in the 0.5% solution died, most likely because of the high concentration of PQQ in the solution, which caused an imbalance of pro-oxidants and antioxidants to too great of an extent, leading to plant death (Rahal et al., 2014). The lower PQQ concentrations did not perform as well as the 0.1% PQQ solution, and therefore the PQQ may not have been present enough to significantly affect plant mitochondria. The mid-range concentration of 0.1% PQQ performed the best when undergoing over-oxidation, and therefore this PQQ concentration is presumed to help the plants resist oxidative stress by strengthening mitochondria, enhancing mitochondrial genesis, and providing antioxidants without overstimulating the organism (Chowanadisai et al.). Though the 0.1% PQQ solution group recorded the best resistance to height reduction, stem color change, and length of survival after oxidative stress, all plants eventually experienced 100% plant death, meaning that 20% H2O2 was too highly concentrated to allow cell damage without inducing cell death.

In conclusion, the results reported herein help to determine if PQQ has a significant influence on the prevention of oxidative stress and the speed of recovery following over-oxidation. Also, the best concentration of PQQ for optimum results can be determined based on the data collected. Plants watered with PQQ solution are hypothesized to respond better to oxidative stress, meaning they will have less lasting damage following H2O2 exposure and will recover at a faster pace compared to the control. Also, the mid-range concentration of PQQ solution is predicted to yield the best results because it will best balance the interplay between pro-oxidants and antioxidants. For future experiments, the mitochondrial death rate should be monitored and this experiment should be carried out in animal species such as mice. Stress can also be tested at different growth stages to determine the effect of oxidative stress on final height. Genetic predispositions for neurodegenerative diseases should also be taken into account in further research.

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