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Chemical oxygen fertilization reduces stress and increases recovery and survival of flooded papaya (*Carica papaya* L.) plants

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A B S T R A C T

In many parts of the world papaya (*Carica papaya* L.) is prone to hypoxic stress due to soil flooding as a result of severe storms or hurricanes. Two experiments were conducted to test the effects of root zone hypoxia on physiology, recovery and survival of papaya and to determine if negative impacts of root hypoxia can be reduced by chemically enriching the root zone with oxygen. In Experiment 1, seedlings in soil were divided into three flooding treatments: (1) 100% of roots submerged, (2) ~75% of roots submerged, or (3) non-flooded; and three oxygen fertilization treatments: (1) 0 g CaO_2, (2) 2.28 g CaO_2 g, or (3) 4.57 g CaO_2 applied to the soil prior to flooding. In soil, CaO_2 is broken down to H_2O_2 which then releases oxygen to the rhizosphere. Therefore, in Experiment 2, plants in a hydroponic solution were divided into six treatments: (1) aeration of the hydroponic solution and no H_2O_2 added to the solution, (2) no aeration and no H_2O_2 added; (3) no aeration and 200 μl of 3% H_2O_2 l^{-1} added daily, (4) no aeration and 500 μl of 3% H_2O_2 l^{-1} added daily, (5) no aeration and 1000 μl of 3% H_2O_2 l^{-1} added daily, or (6) no aeration and 2000 μl of 3% H_2O_2 l^{-1} added daily. In soil, flooding of ~75% or 100% of roots for two days decreased net CO_2 assimilation (A), stomatal conductance (g_s), the leaf chlorophyll index, and the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm). After plants were unflooded, these variables recovered to levels similar to those of the non-flooded treatment for plants with ~75% of the roots submerged but did not recover in plants with 100% of the roots submerged if no CaO_2 was applied to the soil. If 2.28 or 4.57 g of CaO_2 was applied to the soil, A, g_s, leaf chlorophyll index, and Fv/Fm recovered to values similar to those of non-flooded plants. Addition of CaO_2 to the soil also minimized reductions in leaf, stem, root and plant dry weights and increased survival of plants with 100% of roots submerged. For plants in the hydroponic solution, A and g_s were generally lower in the non-aerated treatments than in the aerated treatment. If 500 or 1000 μl H_2O_2 l^{-1} was added to the solution, A of plants in the non-aerated solution tended to recover to levels similar to those of plants in the aerated solution. Root ADH activity tended to be greater in the non-aerated treatment with no H_2O_2 added to the solution than in any of the treatments with H_2O_2 added. This study demonstrated that chemical oxygen enrichment of the root zone reduces flooding stress and increases recovery and post-flooding survival of papaya.

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1. Introduction

In several areas throughout the world, plants are subjected to low soil oxygen concentration in the root zone as a result of flooding due to severe storms or hurricanes (Schaffer, 1998; Schaffer et al., 1992). Flooding events are expected to increase globally as a result of climate change (IPCC, 2014). Papaya (*Carica papaya* L.) is considered sensitive to low soil oxygen content (Balerdi et al., 2005). In a preliminary study, net CO_2 assimilation (A) and stomatal conductance of H_2O (g_s) of papaya decreased after one day of flooding and continued to decline until plants were unflooded (Rodríguez et al., 2014). When the entire root system of papaya plants was completely submerged in water for more than two days, 11 days after they were unflooded plants permanently wilted (Rodríguez et al., 2014).

Oxygen deficiency in the root zone (hypoxia) occurs at soil concentrations lower than 2 mg O_2 l^{-1} H_2O, although the O_2 concentration at which plants become hypoxic differs among plant species (Gibbs and Greenway, 2003). For example, roots of avocado (*Persea americana* Mill.) withstood oxygen levels of 1 mg O_2

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Table 1
Dissolved oxygen content in Krome very gravelly soil solution for plants with ~75% or 100% of roots submerged in H_2O, one and two days after flooding treatments were initiated.

Treatment	~75% of roots submerged		100% of roots submerged	
	Dissolved O_2 concentration (mg l^{-1})		Dissolved O_2 concentration (mg l^{-1})	
	Day 1	Day 2	Day 1	Day 2
0 g CaO_2	5.10 ± 0.41c[a]	5.52 ± 0.24b	3.63 ± 0.92b	4.38 ± 0.89b
2.28 g CaO_2	5.55 ± 0.26b	3.56 ± 0.40ba	7.00 ± 0.76a	5.38 ± 1.70ba
4.57 g CaO_2	6.28 ± 0.28a	6.06 ± 0.37a	8.03 ± 1.09a	7.19 ± 1.50a

[a] Data represent means ± 1 standard deviation of 5 replications per treatment. Different letters indicate significant differences ($P \le 0.05$) among CaO_2 treatments according to a Waller-Duncan K-ratio Test.

l^{-1} H_2O in a hydroponic solution for 10 days with no root damage, whereas concentrations below 1 mg O_2 l^{-1} H_2O or complete lack of O_2 in the solution (anoxia) resulted in root damage (Curtis, 1949).

There has been a considerable amount of research on physiological responses of plants to root hypoxia or anoxia. Among the earliest plant responses to low root zone oxygen content as a result of flooding are reductions in A and g_s (Schaffer et al., 1992). These reductions in A and g_s have been observed before visible stress symptoms occur. Thus, leaf gas exchange measurements have been useful for quantifying stress in response to soil hypoxia or anoxia caused by flooding of the root zone. Flooding has also been observed to negatively affect leaf chlorophyll content (Mielke and Schaffer, 2010) and the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) (Else et al., 2009; Mielke and Schaffer, 2010), an indication of damage to Photosystem 2 (Krause and Weis, 1991).

Lack of oxygen in the rhizosphere can inhibit biochemical and physiological functions in plants. Some plant species can tolerate hypoxia in the root zone for several days whereas others tolerate root zone hypoxia for less than a few hours (Vartapetian and Jackson, 1997). The difference in tolerance to low root zone oxygen concentrations is due to variations in anatomical, morphological or metabolic responses among plant species (Vartapetian and Jackson, 1997). When oxygen content of the rhizosphere is low, a shift occurs from aerobic to anaerobic respiration (Chan and Ronald, 1992). There are two types of anaerobic respiration, fermentation and lactic acid respiration. As a result of root zone hypoxia or anoxia, lactic acid respiration can lead to a build-up of lactic acid, thereby reducing the pH of the cytoplasm (Davies et al., 1974). The resulting cytoplasmic acidosis often leads to cell death. Plants avoid cytoplasmic acidosis by shifting from lactic acid respiration to alcohol respiration with ethanol as the end product of the fermentation pathway (Roberts et al., 1982).

During fermentation, acetaldehyde (the biochemical precursor to ethanol) is much more toxic to plant cells than ethanol and may result in cell death during anaerobic metabolism (Drew, 1997; Vartapetian and Jackson, 1997). The alcohol dehydrogenase (ADH) enzyme catalyzes the reduction of acetaldehyde to ethanol. When oxygen concentration in the root zone is low, ADH activity can increase. For example, flooded *Trifolium subterraneum* plants had 30-fold greater ADH activity than non-flooded plants (Francis et al., 1974). Increased ADH activity can improve a plant's tolerance to hypoxia or anoxia (Chung and Ferl, 1999; Gibbs et al., 2000; Kato-Noguchi, 2000; Morimoto and Yamasue, 2007; Preiszner et al., 2001). For example, *Trifolium repens* plants with high ADH activity under flooding stress showed greater flooding tolerance than plants with low ADH activity (Chan and Ronald, 1992). Thus ADH activity in flooded roots can be used as an indicator of potential flood tolerance.

Chemical oxygen fertilization of the root zone, with slow release (solid) formulations such as calcium peroxide (CaO_2) or magnesium peroxide (MgO_2), or fast release (liquid) formulations such as hydrogen peroxide (H_2O_2) or carbamide peroxide ($CH_4N_2O{\cdot}H_2O_2$), is a potential method of alleviating root hypoxia (Liu and Porterfield, 2014; Liu et al., 2012, 2013). Injecting H_2O_2 into the irrigation water increased oxygen in the soil (Gil et al., 2009a) as well as water use efficiency and biomass of avocado (Gil et al., 2009a). Hydrogen peroxide decomposes in the soil, releasing O_2 which is needed for aerobic metabolism in the roots (Gil et al., 2009a,b). When H_2O_2 comes in contact with water, it reacts to give off 0.5 mol of O_2 per mole H_2O_2 as shown in the equation $H_2O_2 + H_2O \rightarrow 0.5O_2 + 2H_2O$ (Gil et al., 2009a). In soil, solid oxygen compounds (i.e., CaO_2, MgO_2) breakdown to H_2O_2 which then provides oxygen to the rhizosphere (Liu and Porterfield, 2014). Application of MgO_2 to the soil prior to flooding increased flooding tolerance of chrysanthemum by increasing A, lowering the intercellular CO_2 concentration in the leaves, and increasing root dry weight (Wang and Yeh, 2015). Thus, amending soil with slow release solid oxygen compounds such as MgO_2 has the potential to reduce stress caused by low oxygen concentration in the root zone.

The objectives of this study were to test the hypotheses that: (1) plant stress and damage caused by flooding a portion of, or the entire root system of papaya can be reduced or alleviated by amending soil with CaO_2, a slow release solid formulation; and (2) adding H_2O_2 (the breakdown product of CaO_2 in soil) to a hydroponic solution can reduce stress of papaya plants caused by low oxygen in the root zone.

2. Materials and methods

2.1. Study site and plant material

Two experiments were conducted in a greenhouse at the University of Florida, Tropical Research and Education Center in Homestead, Florida (25.5°N latitude and 85.5°W longitude). In the first experiment (Experiment 1) plants in soil in containers were used to test physiological plant responses to flooding and soil applications of CaO_2. The second experiment (Experiment 2) was conducted in a hydroponic solution to test: (1) physiological plant responses to different concentrations of H_2O_2 (the breakdown product of CaO_2 in the soil) and (2) the effects of H_2O_2 on ADH activity of papaya. Growing plants in a hydroponic solution allowed for sampling of root tips free of soil damage because intact, non-damaged root-tips are necessary for accurate determination of root ADH activity.

At the top of the canopy, plants in the greenhouse received 80% of outside photosynthetic photon flux (PPF) as determined with a quantum sensor and LI-1000 light meter (LiCor Instruments, Lincoln, NE, USA). In each experiment, air temperature in the greenhouse was monitored and recorded with a Hobo Pro v2 logger (Onset Computer, Bourne, Massachusetts, USA). During the first experiment, daily air temperatures ranged from 23.1 °C to 36.7 °C with a mean of 23.7 °C. During the second experiment, air temperature ranged from 24.4 °C to 32.1 °C with a daily mean of 27.1 °C.

In each experiment, papaya (*C. papaya* L. cv. Red Lady) seeds were soaked in tap water for 24 h and then sown in flats containing Promix® potting medium (Premier Tech, Quebec, Canada).