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Leaf tissue pigments and chlorophyll fluorescence parameters vary among sweet corn genotypes of differential herbicide sensitivity

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ABSTRACT

Herbicide applications are meant to eliminate weed competition; however, herbicides may also impose abiotic stress on registered crops. Leaf tissue carotenoid pigments play vital roles in the photoprotection of photosynthetic membranes and contribute to non-photochemical quenching (*NPQ*) of excitation energy, both important to plant environmental stress tolerance. Our research objectives were to characterize leaf tissue pigments and chlorophyll fluorescence parameters following post-emergence herbicide applications (simulating an abiotic stress) to sweet corn (*Zea mays* var. *rugosa*) genotypes of differential herbicide sensitivities. Post-emergence herbicide applications of combinations of mesotrione (105 g ai/ha) and atrazine (560 g ai/ha) were applied to 'Merit' (sensitive), 'Temptation' (tolerant), and 'Incredible' (moderately sensitive) sweet corn genotypes. Leaf tissues were sampled after herbicide applications and measured for chlorophyll fluorescence parameters, and the same tissues were analyzed for carotenoid and chlorophyll pigments. Leaf pigments and chlorophyll fluorescence were not affected by any herbicide treatment; however, data revealed significant differences between genotypes for leaf tissue antheraxanthin, β-carotene, zeaxanthin, chlorophyll *a*/*b* ratios, and for values of F_o, F_m, F_v, and *NPQ*, with 'Merit' leaf tissue having higher values than the other two genotypes evaluated. Results demonstrate that genotypic sensitivities to certain post-emergence herbicides may be related to concentrations of photo-protective carotenoids in sweet corn leaf tissues.

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1. Introduction

Stress is a term used to collectively describe numerous conditions that can have negative impacts on plant performance, such as drought, elevated temperature, nutrient imbalances, and many biotic factors. The production of antioxidant compounds within plants can increase or decrease in response to various forms of abiotic environmental stress. Plant secondary metabolites, such as carotenoids, serve functional roles to overcome the negative consequences to plant growth and development caused by stress [1]. Chlorophyll fluorescence measurements have become an effective and widely adapted technique to quantify photosynthetic performance of plants under various stress conditions [2–5]. Moreover, chlorophyll fluorescence parameters have recently been used to assess herbicide efficacy on different crop species [6–8].

Pesticides are important for efficient food and fiber production in modern agriculture; however, herbicide applications may impose stress on registered crops. Herbicide metabolism in plants is divided into conversion, conjugation, and compartmentalization phases. It is during the first phase of conversion that herbicides undergo oxidation and hydroxylation, which usually decreases the phytotoxicity of the active molecule. Cytochrome P450s are monooxygenases involved in the initial conversion metabolism of herbicides [9,10]. Cytochrome P450s are also essential for carotenoid biosynthesis in leaf tissues. Lutein is the most abundant xanthophyll carotenoid in photosynthetic tissues and is essential for light harvesting and photoprotection. Lutein is formed via additions of hydroxyl groups to α-carotene catalyzed by β- and ε-hydroxylases. Recently, it was discovered that the *LUT1* locus in *Arabidopsis* encodes a cytochrome P450-type monooxygenase essential for hydroxylase activities [11].

A total 94,860 hectares of sweet corn were harvested commercially in 2007 in the US, with a total production value of US$625 million [12]. Mesotrione is a carotenoid biosynthesis inhibitor (CBI) currently labeled for pre-emergence and post-emergence control of broadleaf and grass weeds in sweet corn (*Zea mays* var. *rugosa*) production [13]. Sweet corn is tolerant to mesotrione applications; however, differing degrees of sensitivity exist among genotypes [14]. Previous research demonstrated that 'Merit' sweet corn had much higher rates of leaf tissue visual injury following

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early- and late-post application of mesotrione, atrazine, or mesotrione + atrazine than genotypes 'Incredible' or 'Temptation' [15]. Herbicide efficacy studies involving 'Merit' have lead researchers to conclude that this genotype lacks sufficient cytochrome P450s to rapidly metabolize herbicides, and that it is recessive for a single-gene oxidase enzyme vital for rapid herbicide metabolism [16,17]. Although P450 is also essential to both herbicide metabolism and carotenoid biosynthesis, minimal data has been reported regarding carotenoid and chlorophyll pigments and chlorophyll fluorescence parameters following post-emergence herbicide applications to sweet corn genotypes differing in herbicide sensitivity. Our research objectives were to characterize leaf tissue carotenoid and chlorophyll concentrations, and chlorophyll fluorescence parameters following post-emergence herbicide applications to sweet corn genotypes of different herbicide sensitivities ['Merit'(sensitive), 'Temptation'(tolerant), 'Incredible'(moderately sensitive)], based on allelic combinations affecting mesotrione tolerance [18].

2. Materials and method

2.1. Plant culture for sweet corn

'Merit', a yellow-kernel sensitive genotype (Willhite Seed Inc., Poolville, TX); 'Temptation', a bicolor tolerant genotype (Welter Seed & Honey Co., Onslow, IA); and 'Incredible', a yellow-kernel moderately sensitive genotype (Main Street Seed and Supply, Co., Bay City, MI) were used in the study. Herbicide sensitivities were based on visual leaf bleaching following mesotrione applications [14]. 'Merit' is a *su* genotype homozygous for the *sugary1* (*su1*) mutation. 'Merit' will accumulate more sugar and water soluble starch in endosperm tissues than normal field corn at maturity. 'Both 'Incredible' and 'Temptation' are *se* genotypes bred for superior eating quality. The *sugary enhancer1* (*se1*) endosperm mutation modifies the normal *su1* gene and results in greater sucrose accumulation, greater retention of kernel sucrose, and less accumulation of starch compounds [19]. Sweet corn genotypes were seeded at the East Tennessee Research and Education Center in Knoxville, TN (lat. 35° 57′N) on 01 May 2008 in a randomized complete block split-plot design with 4 replications. Sweet corn genotypes acted as the main plots and post-emergence herbicide treatments acted as sub-plots. Seeds were drilled 2.5 cm deep in a Sequatchie silt loam soil (fine-loamy, sili-ceous, thermic, Humic Hapudult) at spacing of 25 cm within rows and 76 cm between rows. Each plot consisted of 4 rows of corn 9.1 m in length. Pre-emergence applications were made to all plots using *s*-metolachlor (Dual Magnum®, Syngenta Crop Protection, Inc., Greensboro, NC) at 1070 g ai/ha and atrazine (Aatrex®, Syngenta Crop Protection, Inc., Greensboro, NC) at 1120 g ai/ha. The insecticide lambda-cyhalothrin (Warrior®, Syngenta Crop Protection, Inc., Greensboro, NC) was applied pre-plant at 32 g ai/ha. Post-emergence herbicide treatments included: (1) untreated control, no post-emergence application; (2) mesotrione (Callisto®, Syngenta Crop Protection, Inc., Greensboro, NC) at 105 g ai/ha as an early post (EPOST); (3) mesotrione at 105 g ai/ha + atrazine at 560 g ai/ha as EPOST; (4) atrazine at 560 g ai/ha as EPOST; (5) mesotrione at 105 g ai/ha as a late post (LPOST); (6) mesotrione at 105 g ai/ha + atrazine at 560 g ai/ha as LPOST; and (7) atrazine at 560 g ai/ha as LPOST. Herbicide treatments were applied as EPOST to corn 5–10 cm tall on 17 May 2008, and as LPOST to corn 15–20 cm tall on 30 May 2008.

2.2. Leaf tissue collection

Leaf tissues were sampled from the sweet corn plants on 12 June 2008. Collections were made 27 days after EPOST treatments and 14 days after LPOST treatments. In order to determine any residual impacts of the herbicides on pools of leaf tissue carotenoids and chlorophyll fluorescence parameters, our goal was to sample tissues when initial herbicide visual injury had subsided. A fully expanded leaf from the 6th leaf whorl was sampled from one representative corn plant per plot. The entire leaf was sampled down to the stalk. Sampling was done at 0900 h when the ambient air temperature was 24.1 °C, the relative humidity was 79.3%, and the light intensity was 421 μmol m^{-2} s^{-1}. Immediately after sampling, leaf tissues were placed in plastic bags and stored in a dark cooler with ice for transport to the lab for chlorophyll fluorescence and pigment analysis.

2.3. Chlorophyll fluorescence measurements for corn leaf tissues

After a dark acclimation period of 60 min, a 20-cm section from the center of each leaf was measured for chlorophyll fluorescence parameters using a pulse-amplitude-modulated (PAM) fluorimeter (Open-FluorCam FC 800, Photon Systems Instruments, Brno, Czech Republic). Measurements started with dark-adapted leaf tissues characterized by a low, minimum fluorescence emission signal (F_0). Leaf tissues were then exposed to a strong flash of light (3000 μmol·m^{-2}·s^{-1} for 0.1 μs) that transiently reduces plastoquinone pool and the primary quinone acceptor, Q_A. The quenching of the reaction centers is eliminated and the fluorescence yield reaches its maximum (F_M). The variable fluorescence (F_V) signal is divided by F_M and used to calculate the maximum quantum yield of photosystem II (PSII) photochemistry QY_{max} (or F_V/F_M). A dark relaxation period following the saturating flash lead to a re-oxidation of the plastoquinone pools. The decline of a fluorescence signal from the F_M level back to F_0 dark-adapted level reflects kinetics at which the reaction centers re-open. The computer program decreased the light intensity of a series of saturating flashes to eliminate transient photochemical quenching by reducing the plastoquinone pool and the Q_A acceptor. The series of saturated flashes allowed the computer program to determine the dynamics of non-photochemical quenching (*NPQ*) during light adaptation [20]. The PAM fluorescence parameters are defined in Table 1. Fluorescence imaging of non-photochemical quenching (*NPQ*) in sweet corn (*Zea mays* var. *rugosa*) leaf tissues of 'Merit', 'Incredible', and 'Temptation' representing sensitive, moderately sensitive, and tolerant genotypes to mesotrione applications appear in Fig. 1.

2.4. Carotenoid and chlorophyll determination for corn leaf tissues – extraction

All leaf tissues samples were frozen at −80 °C prior to lyophilization (Model 6L FreeZone, LabConCo, Kansas City, MO). Pigments were extracted from freeze-dried tissues according to Kopsell et al. [21] and analyzed according to Kopsell et al. [22]. A 0.1 g tissue subsample was re-hydrated with 0.8 mL of ultra pure H_2O and placed in a water bath set at 40 °C for 20 min. After incubation, 0.8 mL of the internal standard ethyl-8′-apo-β-caroten-8′-oate (Sigma Chemical Co., St. Louis, MO) was added to determine extraction efficiency. The addition of 2.5 mL of tetrahydrofuran (THF) was performed after sample hydration. The sample was then

Table 1
Chlorophyll fluorescence parameters of the pulse-amplitude-modulated (PAM) fluorimeter.

Photochemical and non-photochemical quenching parameters		
F_0	=Minimum fluorescence signal	
F_M	=Maximum fluorescence signal	
F_V	=Variable fluorescence signal	$=F_M - F_0$
QY_{max}	=Maximum quantum yield of photosystem II	$=F_V / F_M$
NPQ	=Non-photochemical quenching	$=F_M - F_M\_Ln / F_M$