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Differential responses of *Brachypodium distachyon* genotypes to insect and fungal pathogens

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ABSTRACT

To increase understanding of the interactions between *Brachypodium distachyon* (purple false brome) and its pathogens, six diploid and two hexaploid Plant Introductions (PI) lines were assessed for their resistance/susceptibility to nine economically important fungal pathogens and two species of insect pests affecting closely related grass species. Naturally occurring variation in resistance was found, with two lines being the most resistant and one line being the most susceptible to most of the insects and pathogens tested. Evidence was found for differential activation of key genes in pathogen defense response pathways between susceptible and resistant lines.

1. Introduction

Studies of the molecular biology and genetics of plant resistance have provided much information on how plants recognize and fight invading organisms. The use of *Arabidopsis thaliana* as a model plant in such studies has made a major contribution to our understanding of plant–biotic stress interactions [1]. However, *A. thaliana* is not an ideal model organism for testing responses of grasses to pests and pathogens. For one thing, its cell wall architecture is completely different from that found in grasses. Since the cell wall is the plant's first barrier to invading insects or pathogens, this is an important consideration.

Brachypodium distachyon (L) Beauv., purple false brome, is a winter annual grass (Poaceae) being widely used as a model plant for temperate grasses because of its small genome, short life cycle, and small size [2,3]. It has been proposed for use as a model in studies of specific plant–pathogen or plant–pest interactions involving rice [4,5], wheat [5–8], and barley [8]. However, there are many cultivated grasses important in the turfgrass, animal, and biofuel industries, among others, for which plant–pathogen/pest interaction models still need to be established. If *Brachypodium* is to be used as a model, much more information about the pathogen and pest responses of different genotypes of *Brachypodium* is needed.

The Poaceae family—which includes crucial cultivated crops such as wheat, maize, rice, and barley—can be attacked by several serious insects and pathogens. Major insect pests include the fall armyworm (FAW, *Spodoptera frugiperda* Smith), a "tissue-feeding" insect that attacks a wide variety of grasses [9], and "phloem-feeding" insects such as the Russian wheat aphid (RWA, *Diuraphis noxia* Kurdjumov) [10], which has dramatically reduced the yield of wheat and barley in the United States since its invasion in 1986.

Pathogens on grasses cause a wide range of serious problems including blast disease [11,12], foliar disease [13,14], seed rot [15], damping off [15,16], "dollar spot" [17], and root necrosis [18–20]. In addition to damaging or killing crops, fungal pathogens may also produce metabolites such as mycotoxins that are harmful to humans and livestock that consume affected plant tissue.

Resistance mechanisms in grasses are complex and may involve constitutively expressed primary and secondary metabolites and induced metabolites such as phytoalexins, ethylene, and H_2O_2 accumulation [21–23]. The metabolites jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) are produced in response to attack by insects or pathogens [24,25]. These metabolites aid plant defense in a variety of ways. For example, ethylene (ET) elicits H_2O_2 production, and along with peroxidase (POX2) leads to increased ferulate dimerization and strengthening of the cell wall, enhancing the wall's function as a barrier to potential invaders.

Plants also produce reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to the production of pathogenesis-related (PR) proteins [26]. Among PR proteins, peroxidases have

Abbreviations: FAW, fall armyworm; RWA, Russian wheat aphid; SA, salicylic acid; JA, jasmonic acid; ET, ethylene; ACC, 1-aminocyclopropane-1-carboxylic acid; 4CL, 4-coumarate:CoA ligase; COI1, coronatine-insensitive protein 1; LOX3, lipoxygenase 3; NPR1, non-expressor of pathogenesis-related genes 1; POX2, peroxidase; SAM-DC, S-adenosyl methionine.

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been shown to be effective against pathogens [27] and insect attack [28–31]. Class III plant peroxidases are known to participate in a broad range of physiological processes, such as lignin and suberin formation and cross-linking of cell wall components [26].

In this study, we compare the resistance/susceptibility of eight *Brachypodium* lines to nine economically important fungal pathogens (*Magnaporthe grisea*, *Colletotrichum cereale*, *Sclerotinia homoeocarpa*, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Magnaporthe poae*, *Ophiosphaerella agrostis*, *Ophiosphaerella korrae*, and *Gaeumannomyces graminis*) and two insect pests (fall armyworm, *S. frugiperda*, and Russian wheat aphid, *D. noxia*) affecting closely related grass species. We found natural variation in resistance among the *Brachypodium* genotypes. We further provide evidence for differential activation between susceptible and resistant accessions of key genes of response pathways that are often activated during pathogen attack.

2. Material and methods

2.1. Plant material

Eight *B. distachyon* accessions of different origin and ploidy levels, as listed in Table 1, were included in this study. Two of these were the sequenced PI Bd21 and the inbred line derived from this PI, Bd21-3 [2]. Seeds were supplied by Vicki L. Bradley, USDA, ARS, WRPIS, Washington State University.

2.2. Pathogen screening of B. distachyon genotypes

2.2.1. Plant growth conditions

Seeds of the eight *B. distachyon* accessions were germinated on humidified paper towels in 24-well cell culture plates from CellStar®. Germinated seeds were transferred to 3.5″ or 4.5″ diameter pots (depending on the experiment) containing 5:1 mixture of Miracle-Gro Potting Mix and vermiculite. These seeds were grown in a controlled greenhouse at 24/18 °C (day/night) temperature and a 16-h photoperiod, for six to eight weeks prior to FAW screening, except for genotype CWC, which was grown for four weeks. Plants were fertilized at two-week intervals with a solution containing 1.1 g l^{-1} N, 0.28 g l^{-1} P and 1.0 g l^{-1} K and watered regularly.

2.3. Insect performance on Brachypodium leaves

2.3.1. FAW—source of insects

Eggs of the maize host strain of the fall armyworm (FAW, *S. frugiperda*) were supplied by the USDA-Corn Insect Research Facility at Mississippi State University, through Dr. Dawn Luthe (PSU). Newly hatched caterpillars were reared on a wheat germ- and casein-based diet [32]; ingredients were purchased from BIOSERV (Frenchtown, NJ, USA). Caterpillars were kept individually in diet cups in an incubator at 27 °C and 16 h of light [33] for approximately four days, until they reached their second instar, before being used for bioassays. Bioassays were conducted with larvae of approximately the same stage and weight.

2.3.2. FAW—larval growth performance

Second-instar larvae were placed on one of seven particular diet treatments, held in individual diet cups containing 1% agarose (to avoid drying of the leaf material). Diet treatments consisted of fully developed leaves of the different *B. distachyon* genotypes, assayed in one, two, or three trials, depending on the genotype. For each treatment, 15 larvae were used. Larvae were allowed to feed on fresh leaf tissue, added every other day according to the caterpillars' needs, for ten days. Diet cups were cleaned daily to avoid fungal growth. Cups containing larvae and food were held under laboratory conditions, at 21 °C and a 10:14 day/night photoperiod. Larvae were weighed at the start of the experiment and every other day afterwards to follow their development on the different *Brachypodium* genotypes and on control (maize and fescue) leaves. The data presented here were obtained ten days after starting the bioassay, when FAW caterpillars reached their highest larval weight, and just before pupation on the control host plants (susceptible maize inbred B73) under the experimental conditions.

2.3.3. Control plant growth conditions

Maize (*Zea mays*) inbred lines Mp708, Tx601 and B73—which have been reported previously as resistant, medium susceptible, and highly susceptible, respectively, to the FAW under Pennsylvania conditions [34]—were used as controls, along with *Festuca arundinacea* genotype (20BN3) from the cultivar S170. Maize plants were grown in a Hagerstown loam in a greenhouse with 16 h of light at a temperature of 28/22 °C (day/night) until reaching V7 to V8 stage (four to five weeks after planting); the mid-whorl part of the plant was used for the bioassays. Tall fescue tillers were grown in a controlled environmental chamber at 22/16 °C (day/night) temperatures and a 16-h photoperiod.

2.3.4. Aphids—source of insects

The two biotypes of Russian Wheat Aphid (RWA, *D. noxia*) RWA1 and RWA2 were supplied by the USDA-ARS, Stillwater, OK. These biotypes, previously described as the least (RWA1) and most (RWA2) virulent of this species [10], were maintained individually on the susceptible wheat variety "Yuma" for colony multiplication and insect development. The experiment was set up in cages specifically built for this purpose, using the insect screen LS Econet, from L. Svensson, Inc.®, with light transmission of 84% and 600 × 600 mm hole size. Cages measured 41 cm (L × W × H).

2.3.5. RWA—growth performance

Two replicate (4.5″) pots were used to grow each *Brachypodium* genotype (CWA-CWJ) and wheat control (Yuma) line, and plants were randomly arranged in cages (76 × 50 × 46 cm, $L \times W \times H$) that were placed inside a growth chamber for the duration of the experiment. Plants were fertilized and watered regularly, as previously described. Infestation was made with ~10 aphids, a mix of nymphs and adults, per plant. The experiment was evaluated when wheat plants reached level 8–9 of chlorosis (plants completely attacked), which in this experiment occurred after two weeks. The evaluation was made by visual rating using a scale from 1 to 9, where a rating of 1 was given to a healthy plant and a rating of 9 to a completely dead or leaf-rolling plant [35,36]. However, leaf rolling was rarely observed in these experiments.

Table 1
Brachypodium distachyon genotypes used in this research, nomenclature, plant introduction (PI) accession number, geographic origin and ploidy level.[a]

Nomenclature used in this research	PI accession	Geographic origin	Ploidy
CWA	Bd[b] 21-3	Iraq	Diploid
CWB	Bd 21	Iraq	Diploid
CWC	227011	Iran	Hexaploid
CWD	372787	Uruguay	Hexaploid
CWE	185133	Iraq	Diploid
CWF	245730	Turkey	Diploid
CWG	208216	South Africa	Diploid
CWJ	Bd 3-1	Iraq	Diploid

[a] Vogel et al. [3].
[b] Bd indicates an inbred.