Field Crops Research 216 (2018) 189–196

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Genotype, environment, and genotype by environment interaction for seed isoflavone concentration in soybean grown in soybean cyst nematode infested and non-Infested environments

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ARTICLE INFO

Keywords:
Soybean
Soybean cyst nematode
Genotype by environment interaction
G and GE biplot

ABSTRACT

Soybean (Glycine max [L.] Merr.) is a major isoflavone producing legume. Isoflavones and their derivatives function as phytoalexins that may function in protecting plants against pathogens. They are also known as phytoestrogens in the human diet, which have putative positive human health effects such as reduced risk of breast and prostate cancers, cardiovascular disease, and high blood cholesterol levels. Therefore, increasing isoflavone concentration could be a desirable target for developing added value for food-grade soybean cultivars. Soybean cyst nematode (SCN; Heterodera glycines) is the most yield-limiting pathogen for soybean world-wide. The objectives of this study were to: 1) determine genotype (G) and genotype-by-environment (GE) interaction variation for seed isoflavone concentration and, 2) determine the effects that SCN resistance may have on seed isoflavone concentration in SCN infested and non-infested environments. A population of 109 recombinant inbred lines (RILs), derived from a cross between DH4202 and RCAT1004, along with 11 commercial check cultivars were grown in two SCN infested and two non-infested fields in southern Ontario, Canada, in 2015 and 2016. The genotypes were categorized into resistant, moderately resistant, and susceptible lines based on SCN female indices calculated from SCN infested environments. Significant G, environment (E), and GE interaction effects were observed for total isoflavone concentration as well as for yield. Higher isoflavone concentrations were observed in non-infested environments when compared with SCN infested environments for all three levels of resistance. Within SCN infested environments, resistant genotypes had significantly higher isoflavone concentrations than susceptible genotypes, indicating a potential role of isoflavones in plant defense from SCN in resistant genotypes. SCN infested environments were found to be more reliable for genotype evaluation of isoflavone concentration than non-infested environments due to similar relative performance of genotypes in all SCN infested environments. A strong relationship between the level of SCN resistance and relative yield among the genotypes was observed in SCN environments. When evaluating genotypes, soybean breeders should consider the negative impact SCN has on seed isoflavone concentration, the positive relationship between SCN resistance and isoflavone concentration in SCN environments, as well as the greater stability in isoflavone concentration across genotypes within SCN environments compared to the non-infested ones.

1. Introduction

Soybean (Glycine max [L.] Merr.) is one of the major isoflavone producing members of the Fabaceae family. Isoflavones are synthesized via the phenylpropanoid pathway and found almost exclusively in legume species. Isoflavones and their derivatives are important for plants defense against pathogens and mediating soybean symbiosis with nitrogen-fixing rhizobia (Samac and Graham, 2007). Isoflavones are also associated with positive human health effects such as reduced risk of breast and prostate cancers, cardiovascular disease, high blood cholesterol levels, and prevention of osteoporosis and hot flashes in postmenopausal women (Dastmalchi and Dhaubhadel, 2014). Due to these positive attributes, developing high yielding food-grade soybean cultivars with elevated seed isoflavone concentration has recently attracted much attention.

Soybean cyst nematode (Heterodera glycines Ichinohe; SCN) is considered to have the largest impact on yield among all soybean pathogens globally, including North America (Wrather and Koenning,

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https://doi.org/10.1016/j.fcr.2017.11.021
Received 10 July 2017; Received in revised form 22 November 2017; Accepted 22 November 2017

2006; Koenning and Wrather, 2010). As of 2014, almost every soybean producing state in the United States has been affected by SCN (Tylka and Marett, 2014). In Canada, SCN has been reported in multiple counties in Ontario and in Quebec (Mimee et al., 2014). While no cases of SCN have been reported in the expanding soybean producing provinces of Manitoba and Saskatchewan, there is potential for it to spread into these provinces from northern states (Tylka and Marett, 2014). Therefore, developing soybean cultivars with resistance to SCN is important for soybean production across the world. Isoflavone concentration in soybean is a quantitative trait that is influenced by many genetic and environmental factors. Temperature was shown to have a significant effect on seed isoflavone concentration, with less isoflavones accumulating in high temperatures (Lozovaya et al., 2005). Long term drought over seed development periods have also been associated with lower seed isoflavone content in soybean; however, it is reported that brief severe periods of drought had no significant effect on seed isoflavone levels (Gutierrez-Gonzalez et al., 2010).

Many previous studies have identified significant genotype by environment interaction for isoflavones in soybean (Lee et al., 2003; Primomo et al., 2005; Smallwood et al., 2014; Morrison et al., 2008). Lee et al. (2003) identified significant genotype by environment interactions, and within that interaction the genotype-by-year and genotype–by-year-by-site interactions accounted for most of the variation. Some studies have identified significant GE interaction, but noticed consistent ranking of genotypes with high and low isoflavone concentrations, suggesting that breeding for these phenotypes is possible in any environment (Hoeck et al., 2000; Murphy et al., 2009).

The presence of GE interaction allows for further statistical analysis, which aids researchers in visualizing genotype by environment interaction. Some of the proposed methods for studying GE interaction include regression coefficients (Finlay and Wilkinson, 1963), deviations from regression (Eberhart and Russel, 1966), stability variance (Shukla, 1972), and the additive main effects and multiplicative interaction (AMMI) model (Gauch and Zobel, 1988). The G and GE (GGE) biplot method using sites regression (SREG) analysis, however, is an effective way for multi-environment data to be used to assess genotypic performance across environments and to evaluate test environments (Yan et al., 2000; Yan et al., 2007). This method has been used to evaluate test environments and visualize relationships between traits in soybeans grown in southern Ontario (Yan and Rajcan, 2002). While there are methods of visualizing GE interaction with AMMI, GGE biplots are useful in this study for their effectiveness in evaluating multiple test environments while simultaneously providing information about genotype performance in environments through detecting crossover and non-crossover interactions (Yan et al., 2007; Bernardo 2010).

Due to the increasing problem of SCN, soybean breeders will need to focus on developing high-yielding cultivars with good SCN resistance. It is important to determine any effect SCN, or SCN resistance has on other traits that soybean breeders or producers may be interested in. To our knowledge, no previous published work has determined SCN or SCN resistance's effect on seed isoflavone concentration, or used GGE biplot analysis to examine similarities and differences between SCN infested and non-infested environments. Therefore, the main objective of this study was to study and visualize genotype (G) and genotype by environment (GE) interaction variation of seed isoflavone concentration in a soybean recombinant inbred line population evaluated in SCN infested and non-infested environments.

2. Materials and methods

2.1. Field trials

Field trials consisting of a population of 109 $F_{4:7}$ recombinant inbred lines (RILs) developed from a cross between RCAT 1004 (moderately high isoflavone; SCN-resistant) and DH 4202 (low isoflavone; SCN-susceptible) and 11 commercial check varieties were grown in four locations across southwestern Ontario, Canada, over two years, 2015 and 2016. The locations Rodney (ROD) (42°35′50.43"N, 81°38′32.76"W) and Houston (HOU) (42°24′11.84"N, 82°7′31.54"W) had SCN infested soils, while Chatham (CHT) (42°20′12.76"N, 82°15′21.75"W) and Woodstock (WST) (43°8′44.78"N, 80°47′2.46"W) were non-infested with SCN. The ROD, HOU, and CHT locations had fine sandy loam soil, and WST had loam soil. Each plot consisted of five rows planted 4 m long with a row spacing of 43 cm. The rows were trimmed to 3.8 m in length after emergence and only the inside three rows were harvested. In each plot, 500 soybean seeds were planted to reach a plant density of 54 seeds m^{-2}. Environments were abbreviated by their location ID followed by the growth year (e.g., Woodstock in 2015 was called WST15). Plots were arranged in a randomized complete block design (RCBD) with three replicates in the SCN infested environments, Rodney and Houston, and two replicates in the non-infested locations, Chatham and Woodstock. A summary of monthly weather statistics for each environment is presented in Supplementary Table 1.

2.2. Data collection

Seed yield data was collected for each harvested plot, adjusted to 13% moisture, and is presented in tonne ha^{-1}. Total seed isoflavone concentration was determined by using a near-infrared sprectroscopy (NIRS) method developed at Agriculture and Agri-food Canada (AAFC) in Harrow, ON, Canada. Approximately 30 g of seed from each plot was ground to a fine powder using a FOSS Knifetec™ 1095 sample grinder (FOSS NIRSystems, Inc., Laurel, MD). Grinder components were cleaned between samples to prevent contamination. Ground samples were stored in plastic vials for up to 24 h before NIRS analysis.

Isoflavone concentrations of the ground samples were measured using a FOSS 6500 spectrophotometer (FOSS NIRSystems, Inc., Laurel, MD) and ISIscan software (Intrasoft International, Laurel, MD). The spectra generated for each sample were used to predict isoflavone concentration using a prediction model developed by AAFC in Harrow, ON (Morrison et al., 2008). The total isoflavone values represent the sum of aglycone and conjugated forms, presented as aglycone equivalents on a dry weight basis (Morrison et al., 2008).

SCN resistance for each genotype was generated using SCN count data gathered from the SCN infested locations, Houston and Rodney. Resistance is presented as a female index (FI), based on cyst (female) counts from roots gathered from each plot. The susceptible cultivar Lee 74 was used to calculate the FI for each plot using the following equation:

$$FI = \frac{\#\text{of females on test line}}{\#\text{of females on Lee74}} x100\% \quad (1)$$

2.3. Boxplots and scatterplot matrices

Boxplots for yield and total isoflavones were generated with genotype LSMEANS from each environment to show genotype variation within each environment using PROC UNIVARIATE in SAS 9.4 (SAS Institute Inc., Cary, NC). Scatterplot matrices for yield and total isoflavones were generated using genotype LSMEANS from each environment using the Matrix statement within PROC SGSCATTER in SAS 9.4 (SAS Institute Inc., Cary, NC).

2.4. Analysis of variance

All analyses of variance (ANOVA) were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). ANOVA for each environment was performed to generate LSMEANS using PROC MIXED with genotype as a fixed effect and blocks as a random effect. Combined ANOVA was performed for yield and total isoflavones using PROC MIXED with genotype, environment, and genotype by environment as fixed effects