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Improvement of crop protection against greenbug using the worldwide sorghum germplasm collection and genomics-based approaches

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Abstract

Successful development of new sorghum cultivars and hybrids to ensure sustainable production depends largely on the availability of genetic resources with desirable traits such as pest resistance. Our recent research has focused on improvement of crop protection against greenbugs using the worldwide germplasm collection and genomics-based approaches. First, we conducted the systematic evaluation of a worldwide germplasm collection in order to identify new sources of greenbug resistance. Twenty-one resistant lines were identified, which offered new sources of resistance to sorghum breeding. Molecular markers used to assess the genetic diversity among those resistant lines suggested relatively diverse resistant sources in the sorghum germplasm collection. More recently, a mapping project was executed to associate the resistance genes with sorghum chromosomes. The mapping data indicated one major and a minor quantitative trait loci reside on chromosome 9 and are responsible for resistance to greenbug. In addition, cDNA microarrays were used to monitor greenbug-induced gene expression in sorghum plants. This study has developed a transcriptional profile for sorghum in response to greenbug attack, which provides us with useful molecular information for discovery of greenbug resistance genes and a better understanding of the genetic mechanisms controlling host defences in sorghum.

Keywords: DNA microarray; genomics; greenbug aphid; molecular marker; sorghum

Introduction

Sorghum (*Sorghum bicolor*) is a leading crop grown in more than 100 countries (Frederiksen and Odvody, 2000; U.S. Grains Council, 2006). Grain sorghum is an important staple food crop in Africa, South Asia and Central America. Sorghum is grown as animal feed and for bioethanol production in the USA, Australia and other developed countries. As with most crops, sorghum is the host to many pest insects. Greenbug (*Schizaphis graminum*) is one of the major pests of sorghum as well as wheat and barley worldwide (Hackerott *et al*., 1969; Andrews *et al*., 1993). For example, damage by greenbug to sorghum is estimated to cost US sorghum producers $248 million annually (INTSORMIL, 2006).

One of the most effective and environmentally sound insect pest management approaches is the use of genetically resistant cultivars and hybrids as the core component of an integrated pest management programme. Several examples of successful deployment of resistant cultivars can be cited, but these examples are oftentimes short-lived due to the tremendous diversity

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of biotypes in the target pest populations and evolution of their virulence. New sources of resistance to these key pests must be found continuously and incorporated into high-performance breeding lines for cultivar/hybrid development. Host resistance mechanisms must be characterized, and resistance genes/quantitative trait loci (QTLs) and molecular markers linked to these traits need to be identified in order to assure movement into commercial cultivars and hybrids through various breeding methods and to enable more efficient crop breeding. Furthermore, plant genomics has proven to be a promising new means for crop improvement. This paper takes sorghum–greenbug as the example to demonstrate the current success and future potential in improvement of crop protection against greenbugs using worldwide germplasm collection and genomics-based approaches.

Evaluation of sorghum germplasm for new sources of resistance to greenbugs

Continuous improvement in crop defence is dependent on the availability of diverse genetic resources and judicious use of effective sources of resistance. At present, over 40,000 sorghum germplasm accessions have been captured and conserved in the USDA National Plant Germplasm System (NPGS, 2010). This collection represents the great wealth of sorghum genetics resources and is invaluable to both sorghum research and various crop improvement programmes. However, those genetic materials are of little value unless they are evaluated, documented and utilized. Thus, one of our current research emphases has been the systematic evaluation of the entire US collection of sorghum germplasm to identify new sources of greenbug resistance, and to subsequently characterize the newly identified sources to facilitate breeding sorghum with insect resistance.

In this project, these sorghum germplasm accessions from locations around the world were evaluated for their response to greenbug feeding in the greenhouse at the USDA-ARS Plant Science Research Laboratory, Stillwater, Oklahoma. As a result, 21 germplasm accessions were identified that possessed resistance to the greenbug, as shown in Table 1. When compared with susceptible checks, the newly identified accessions had relatively low greenbug damage scores, indicating their inherent resistance to greenbug. Among these resistant sources, four accessions showed high levels of resistance ranging from 1.0 to 2.8 on a 1 to 6 scale (1 = no damage; 6 = 100% damage). The other 17 accessions demonstrated moderate resistance with resistance ratings from 2.9 to 3.8. These sorghum accessions are resistant to greenbug biotype I and offer new resistance sources to sorghum breeding programmes.

Table 1. Greenbug damage scores of the new sources of sorghum germplasm lines

No.	Seed ID	PI number	Origin	Damage rating[a]
1	1072	221719	South Africa	3.5 ± 0.5bcd[b]
2	2-969	452752	Ethiopia	3.0 ± 1.1de
3	3-416	455203	Ethiopia	3.2 ± 1.2bcde
4	3-722	455512	Ethiopia	3.3 ± 1.0cd
5	3-1022	455812	Ethiopia	3.8 ± 1.0bcd
6	3-1698	456490	Ethiopia	3.5 ± 1.7bcd
7	3-1711	456504	Ethiopia	3.4 ± 1.0bcd
8	3-2419	457212	Ethiopia	3.7 ± 0.8bcd
9	3-2513	457314	Ethiopia	3.1 ± 1.1cde
10	4-2683	482903	Zimbabwe	3.5 ± 1.3bcd
11	5-462	500963	Zambia	3.5 ± 0.6bcd
12	6-384	515888	Togo	3.6 ± 1.4bcd
13	7-855	535779	USA	2.0 ± 0.7efg
14	7-943	536594	Honduras	3.3 ± 1.0cd
15	7-1859	545501	Sudan	2.8 ± 0.5de
16	7-2535	560387	South Africa	3.8 ± 0.9bcd
17	8-17	562891	India	3.5 ± 1.1bcd
18	9-99	585393	Nigeria	3.3 ± 0.7bcde
19	9-2983	591008	USA	3.8 ± 1.6bcd
20	10-562	596542	USA	2.6 ± 1.8def
21	10-1231	607900	USA	1.1 ± 0.1g
22	Resistant	550607	China	2.0 ± 0.4efg
23	Susceptible	Westland A line	USA	5.8 ± 0.2a

[a] Based on greenbug damage to the sorghum seedlings, scores were recorded on a 1–6 scale, where 1 = ≤ 20%, 2 = 20–40%, 3 = 40–60%, 4 = 60–80%, 5 ≥ 80% damage and 6 = plant death.
[b] Means with the same letter are not significantly different at probability level of 0.05.

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Gene discovery through gene expression profiling

Identifying what genes are expressed in a particular tissue or at a specific time is often very useful to determine their function. Recently, genomics techniques such as DNA microarrays, large-scale gene expression profiling (transcriptome) and associated bioinformatics analysis make it possible to monitor expression of all genes simultaneously in any given tissue or following a specific treatment (Alba *et al*., 2004).

Using cDNA microarrays, we have recently examined the transcriptional changes in sorghum seedlings and compared the results from parallel systems, greenbug-resistant and -susceptible genotypes, leading to the detection of differentially expressed transcripts during infestation by greenbug biotype I, corresponding to 157 sorghum genes. The experiments showed comprehensive gene activities resulted from up-regulating or activating existing defence pathways in sorghum seedlings in response to greenbug feeding. Of the aphid-induced genes identified in this study, 38 genes exhibited three-fold or higher abundance in their expression and 26 genes were significantly reduced. For further analysis, the genes that showed differential expression were cloned and sequenced. The resulting sequences were then annotated by comparison with GenBank databases using the BLASTX search program. Sequence similarity searches allowed putative functions to be assigned to 16 cDNA clones/genes (Table 2) that were directly or indirectly involved in host defence against greenbug attack. Our detailed studies also suggested that the defence responses against greenbug in sorghum are coordinately modulated by versatile molecular regulators such as salicylic acid, jasmonic acid, abscisic acid and phytohormones (Park *et al*., 2006).

Although the application of microarray technology to the analysis of plant defence responses is still a very recent approach (Zhu-Salzman *et al*., 2004; Park *et al*., 2006), the initial microarray experiments on studies of plant responses to attack by greenbugs have shown the promise for functional characterization of important processes such as plant defence against aphids. Furthermore, these expression profiling studies completed to date have already identified an amazing number of genes that had not previously been implicated in plant defences against insects. Large sets of informative data were generated that led to the identification of many potential defence-related transcripts. The ability to identify changes in gene expression, particularly in response to disease, insect pest or abiotic stresses is significant since exploration of gene activation in plant defence on a genome-wide scale could be important in discovering novel defence genes or strategies for crop improvement.

Development and application of molecular markers in study of plant defence

The development of DNA markers has facilitated the construction of genetic maps for an economically important plant species. A genetic map depicts the linear arrangement of DNA markers along each chromosome and the genetic distances between adjacent markers. Once the genetic maps are constructed, they can facilitate practical

Table 2. Identification of the defence or defence-related genes that differentially expressed in sorghum seedlings in response to greenbug attack

cDNA ID[a]	Gene product – putative biological function[b]	Fold changes[c]
DR831455	Sulphur-rich/thionin-like protein	13.251
DR831570	β-Glucosidase	3.899
DR831456	Glucan endo-1,3-β-glucanase	2.218
DR831457	S-like RNase	1.801
DR831459	Cysteine proteinase inhibitor	3.324
DR831458	Cysteine proteinase	2.652
DR831460	Polyphenol oxidase	3.573
DR831462	Legumain-like protease	2.105
DR831463	Endo-1,4-β-glucanase	2.621
DR831464	Wound inductive gene	2.621
DR831465	Multiple stress-responsive zinc-finger protein	2.428
DR831466	Oxysterol-binding protein	2.135
DR831467	Cytochrome P450-like protein	2.757
DR831468	Cytochrome P450 monooxygenase	−2.253
DR831470	Xa1-like protein	2.39
DR831471	OTU-like cystein domain-containing protein	−1.066

[a] GenBank accession number. [b] BLASTX research was used to determine homologous genes and putative functions. [c] Values of signal intensity ratios showing up- or down-regulation.

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applications in plant breeding such as the identification of important agronomic traits (in many cases QTLs) and marker-assisted selection. Genetic maps are also an important resource for plant gene isolation through map-based cloning and potential for modification without affecting other important traits.

Recently, simple sequence repeats (SSRs) or microsatellites have become the most important DNA marker technology as they proved to be a more dependable, rapid and inexpensive tool for plant genotyping (Yang *et al*., 1996). We have recently constructed a detailed SSR-based genetic map for sorghum (Wu and Huang, 2007). Using the genetic map and SSR markers, we were able to locate one major and a minor QTL on chromosome 9 that are responsible for resistance to greenbug (Wu and Huang, 2008). The major resistance QTL on chromosome 9, designated as QSsgr-09-01, was found to reside in the interval of 7.3 cM between Xtxp289 and Xtxp358, on the basis of linkage and QTL analyses. QSsgr-09-01 accounted for 54.5–80.3% of the phenotypic variation in genetic resistance to greenbug biotype I. The second QTL identified on SBI-09 was flanked by Xtxp67 and Xtxp230, and designated as QSsgr-09-02. QSsgr-09-02 explained 1.3–5.9% of the resistance variation. With the rapidly increasing availability of cDNA clones and expressed sequence tags (ESTs), we took the *in silico* mining approach to develop EST-SSRs. From the available 25,456 ESTs or cDNA sequences, we have developed 2680 EST-SSRs (Huang, 2008). These newly developed sorghum EST-SSR markers represent an additional resource for genetic mapping, comparative genomics and evaluation of co-location between QTLs and functionally associated markers in sorghum. Among these newly identified markers, 200 selected EST-SSR markers were examined for transferability to related cereal crops and the results showed their potential as molecular markers in maize, sugarcane, rice, wheat and barley.

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