Developing an integrated system for Asian Soybean Rust control

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Dr. Karen Century, Project Manager
BASF – We create chemistry

- Our chemistry is used in almost all industries
- We combine economic success, social responsibility and environmental protection
- Sales 2017: €64,457 million
- EBIT 2017: €8,522 million
- Employees (as of December 31, 2017): 115,490
- 6 Verbund sites and 347 other production sites
Research and Development at a glance

BASF – Champion in the chemical industry

High R&D expenditures

Over € 9 billion annual sales with innovations

Around 3,000 running research projects, 10,000 employees worldwide in R&D

Global Know-How Verbund with external partners

R&D is a major growth driver for BASF

* No.1 in the Patent Asset Index™
Our Mission and Vision

- We strive to provide a better quality of life and improved environment through Plant Science Technologies
- We drive innovative solutions for agriculture, nutrition, and industrial applications, creating value for BASF and customers
- We base our strategy on supporting farmers:
  - Yield Increase & Stress Tolerance
  - Herbicide Tolerance
  - Fungal Resistance
  - Quality traits (EPA+DHA canola oil)
BASF Plant Science Strategy
Competence Center for Plant Technologies

What we do
- We provide cutting-edge plant science services to BASF Operating Divisions to generate value
- We partner with leading seed companies for development and commercialization of traits
- We invest a high double-digit million € amount in research and development every year

How we do it
- Constantly improve our technology platform to push innovations and competence to serve Operating Divisions and customers
Asian Soybean Rust resistant soybean
Solving a huge problem in soybean production

Project overview

» Soybeans provide oil and protein; worldwide >250 million tons are produced
» Soybeans are highly sensitive to Asian Soybean Rust (ASR), which can cause yield losses of up to 90%
» New tools to manage ASR are required urgently
  • Key fungicides lose efficacy quickly as the ASR fungus develops resistance
  • Native resistance genes in soybean also broken down rapidly as the fungus evolves
» Project objective: Provide farmers with a durable system for reliable protection against soybean rust
  • Combining fungicides with biotech solutions will extend the life of both tools
  • Better manage fungicide applications while protecting soybean yield
Fungicide use by target pathogen in Brazil

$1.4 billion total

78% of fungicide applications in Brazil are aimed at controlling Asian Soybean rust

Source: Kleffmann Amis Soybean 13/14
Data (Embrapa) show that fungicides rapidly lose efficacy against ASR over time.

Combining fungicides with genetic solutions will extend the life of both tools while providing growers with a stronger, more durable solution to ASR.
The unusual enemy: Asian soybean rust (ASR) fungus Phakopsora pachyrhizi

› Unusual infection process
  • ASR fungus directly penetrates epidermis (5 MPa = 725psi turgor pressure) and kills first cell before establishing biotrophic interaction

› Unusually wide host range
  • ASR fungus can infect >50 species from various clades of legumes

› Unusually huge genome
  • ASR genome estimated larger than 850 Mb (typical fungi 50-80Mb)

› Unusually minimalistic life-cycle
  • Only a single spore type found in the wild (urediospores) compared to stem rust having five distinct spore types and requires alternate hosts

› Unusually low number of resistance genes
  • Relatively few classical R-genes for ASR resistance have been identified, despite significant effort
Non-Host Resistance

- Soybeans are highly susceptible to ASR
- There are plants with a natural immunity against ASR, like chickpeas, certain clovers, tobacco, Arabidopsis, etc.
- Non-Host Resistance strategy relies on introducing the responsible genes into soybean
- Strong and durable resistance is achieved by combining genes with different mechanisms for resistance
- Broad gene discovery to identify multiple lead genes with different modes of action
Fungal-resistant soybeans
Finding the right genes

Gene discovery based on non-host resistance model

» Broad gene discovery approaches by analyzing genomic diversity of non-host plants on histological and molecular levels

» Hypothesis-driven nominations based on scientific literature and integration of internal and public data

» Collaborations with academic partners
  • Screening of Medicago mutant libraries in collaboration with Noble Foundation
  • Transcriptomic analysis of Arabidopsis mutants after soybean rust infection in collaboration with RWTH Aachen
Fungal-resistant soybeans
Finding the right genes

Transcriptomic analysis of Arabidopsis mutants after soybean rust infection

» Analysis of gene expression to elucidate molecular basis of mesophyll resistance of Arabidopsis pen2 plants against soybean rust fungus

» Selection of genes upregulated in pen2, but not in wild-type (Col-0) or pen2 pad4 sag101 triple mutant

» Interspecies transfer of these genes to soybean leads to increased resistance against soybean rust

Laser Capture Microdissection and RNA sequencing
Understanding the soybean-rust interface

» LCM-RNAseq was applied to identify differentially regulated genes in the interacting plant-fungal cells
» Increased precision and sensitivity in the detection of gene expression in specific cell types
» Our results should reveal key molecular processes in response to fungal infection.
LCM to select ASR-infected soybean samples

Cross-section of infected leaf for Laser Capture Microdissection (LCM)

» Increasing resolution and understanding of the host-pathogen interface
Testing candidate genes in the field in Brazil

80 days after planting, no fungicide
Strong results achieved by gene combinations

- Field trials, 96 days after planting, no fungicide treatment
- Positive results achieved with multiple different gene stacks
Developing an integrated system for rust control

Fungicide “skip-spray” field trial design

- **Goal:** Determine how transgenic events perform under “commercial” fungicide spray regimens
- **Method:** Evaluate transgenic events in combination with different fungicide treatments (skip-spray):
  - Untreated (U), “Commercial” 3 spray (ABC), Skip 1\(^{st}\) (BC), Skip 2\(^{nd}\) (AC), Skip 3\(^{rd}\) (AB)

84 days after planting
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102 days after planting
Resistance to multiple ASR strains

- Detached leaf assay with rust isolates from different regions in Brazil to confirm broad, non-race-specific resistance
- Single transgenic event represented in photos
- Disease percentage (by leaf coverage) given
- Preliminary results show increased resistance to all isolates tested

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<thead>
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<th>Isolate 1</th>
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Summary

**Project objective: Provide farmers with a durable, integrated system for reliable protection against soybean rust**

» Multiple ASR resistance-enhancing lead genes with different modes of action (transcription factors, regulatory proteins, resistance genes, enzymes) discovered

» Strong increased disease resistance achieved by combination of lead genes

» Results confirmed in five consecutive seasons in field trials in Brazil

» Further development ongoing, targeting market introduction in the next decade
Thanks

...to all contributors
...to all collaborators
...to you for your attention
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