Practical Training Exercise

ANALYZING AND MANAGING RISKS IN LIFE SCIENCES RESEARCH

Based on the article by Ejaz, M, et al. “Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties.” Crop Science. 2012; 52: 2638-26481.

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ADVANCING SCIENCE, SERVING SOCIETY

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You may contact the copyright holder at:
1200 New York Ave.
Washington, DC 20002
cstspinfo@aaas.org
1-202-326-6493

This series of case study exercises was developed with input from: Lindsey Marburger, Nisreen AlHmoud, Oussama ben Fradj, Eleanor Celeste, Gwenaële Coat, Cristine Geers, Irene Jillson, Abdulaziz Kaed, Rawan Khasawneh, Fadia Maki, Kimberly Schaub, and Kavita Berger.

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Learning Objectives

1. Develop the skills to think critically about risks and risk mitigation strategies needed in your own scientific environment;

2. Enhance your ability to identify risk management strategies and approaches that minimize identified risks and maintain the high-quality and utility of the scientific activity; and

3. Apply the risk analysis framework to your own or your peers' scientific activities.

Participant Expectations

By the end of this exercise, you will have familiarity with:

1. The definitions of different types of risks associated with laboratory, field, and public health research.
2. The process of risk analysis—risk identification, assessment, management, and communication—including:
   - How to identify and assess risks by considering the possible likelihood and consequences of risks, and the risks versus benefits of a research activity,
   - Strategies for managing risks, and
   - Who, when, and how to communicate risks.
3. How to apply the risk analysis framework to your own scientific activities.

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Ground Rules for Participation

1. Prior to starting this exercise, participants should have read the case study article.
2. Ask the facilitator to clarify questions about the case study article.
3. Focus on understanding and analyzing the diverse risks involved in the research rather than on critiquing the methodologies or research choices of the authors.
4. Interact with one another in a way that encourages open communication and exchange of ideas. For example, listen to everyone's ideas respectfully.
5. You may want to take your own notes to enhance your ability to actively participate in the training activity.

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Biorisk Glossary

These definitions are from the WHO's *Responsible Life Science for Global Health Security: A Guidance Document*.

- Bioethics
- Biorisk
- Biorisk reduction
- Laboratory biosafety
- Laboratory biosecurity
- Dual-use life sciences research
- Research excellence

<u>Additional concepts:</u>

- Protection of human subjects
- Protection of animal subjects
- Responsible research/responsible conduct of research

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Risk Analysis Framework

Your risk review will follow these 4 stages:

1. **Risk Identification**
2. **Risk Assessment**
3. **Risk Management**
4. **Risk Communication**

1. Risk Identification

process by which researchers consider all possible internal, external, and organizational risks.

Asks the question:

- ***What are the possible risks associated with the research?***

2. Risk Assessment

process by which researchers identify needed resources and consider biosafety/biosecurity recommendations.

Also defined as the “process of evaluating the risk(s) arising from a hazard(s), taking into account the adequacy of any existing controls and deciding whether or not the risk(s) is acceptable).” (OHSAS 18001: 2007)

Asks the questions:

- ***How likely are the risks to occur?***
- ***What are the potential consequences if the risks occur?***
- ***Do the risks outweigh the benefits?***

3. Risk Management

process by which researchers consider regulations/guidelines, training, and SOP compliance issues.

Asks the question:

- ***What risk management strategies could minimize the likelihood that the risk will occur or the consequences if the risks occurred?***

Possible strategies: physical barriers, personnel training or vetting, regulations and laws, and/or alternative experiments

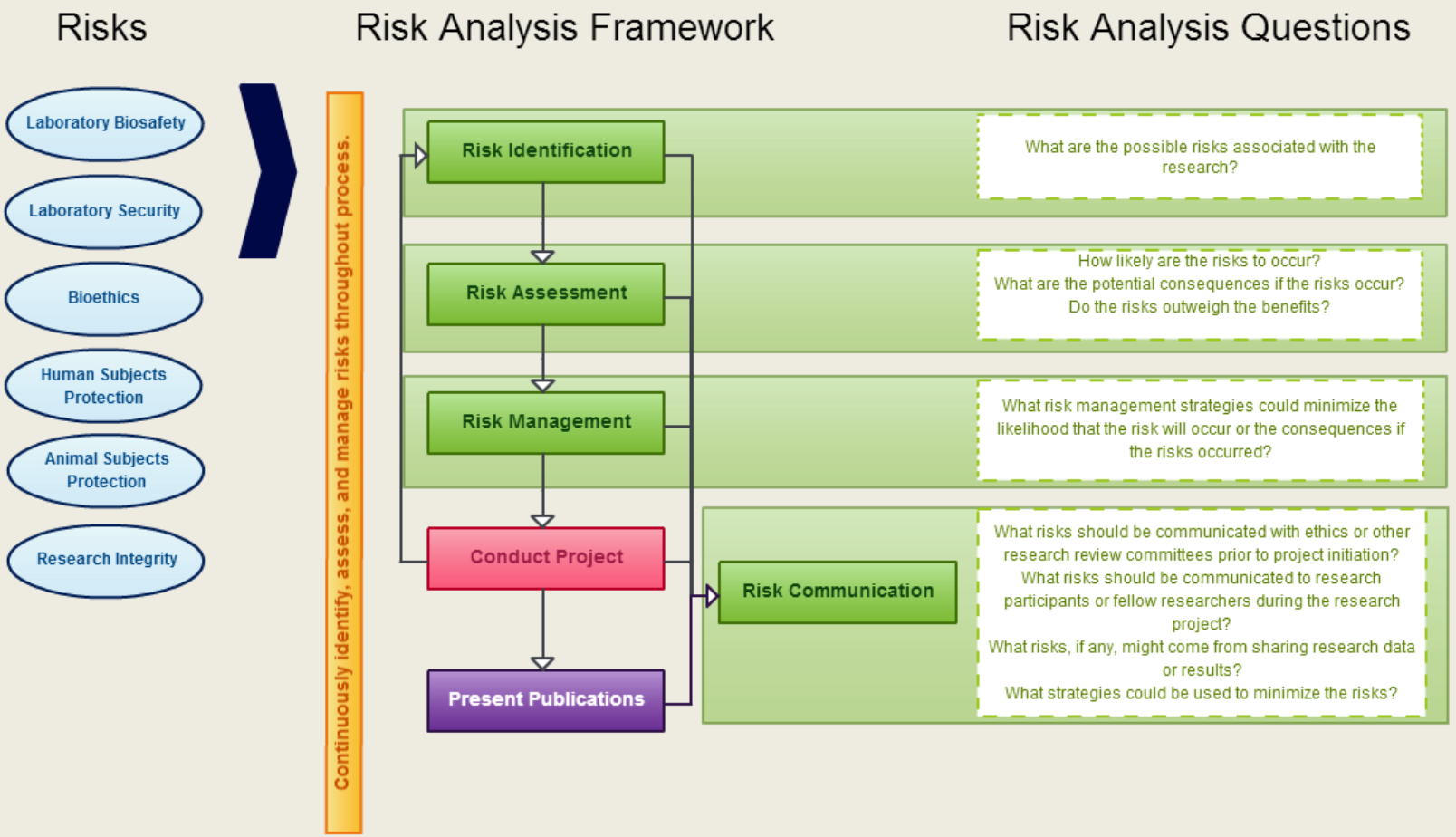
4. Risk Communication

process by which researchers consider communication strategies, non-compliance issues and approval/modification processes.

Asks the questions:

- ***What risks should be communicated with ethics or other research review committees prior to project initiation?***
- ***What risks should be communicated to research participants or fellow researchers during the research project?***
- ***What risks, if any, might come from sharing research data or results?***
- ***What strategies could be used to minimize the risks?***

Risk Analysis Chart



CASE STUDY

Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties

Ejaz, M, et al. “Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties.” Crop Science. 2012; 52: 2638-2648.

Outline of Case Study

Part 1: Research Question/Hypothesis

Part 2: Background Information Overview

Part 3: Research Methodology

Part 4: Risk Analysis in the Research Article

Part 5: Research Results and Conclusions

Research Question/Hypothesis

Research Statement:

Stem rust is a plant disease caused by the pathogen, *Puccinia graminis* f. sp. *tritici*. The pathogen infects common wheat plants and its closest relatives, and causes 50% yield losses.

Several molecular makers are associated with stem rust resistance genes. The use of molecular markers to screen for disease resistance genes in wheat can be used to assist seed selection and breeding.

Information about the presence or absence of stem rust resistance genes in Pakistani spring wheat is limited.

The authors propose to identify the major resistance genes in Pakistani spring wheat varieties using DNA markers of stem rust wheat resistance genes.

Background Information Overview

Wheat Stem Rust

- Wheat stem rust was developed into a biological weapon by the U.S. in the 1960s, Soviet Union, and Iraq in the 1980s.
- Using a broader definition of biosecurity, protection of wheat from stem rust is economically important because diseases can affect crop yield, quality, and/or trade and commerce.
- The virulence of stem rust pathogens results in concerns about food security.

Background Information Overview

Ugg 99

- The Ug99 stem rust strain causes up to 90% yield loss. Although Ug99 has not yet emerged in Pakistan, it might spread to Pakistan through Iran. Some stem rust strains in the Sindh and southern Punjab provinces are becoming more virulent.
- The Ug99 strain first emerged in Uganda in 1999 and spread throughout East Africa, Yemen, Sudan, and Iran. It has been predicted to spread to North Africa, the Middle East, Asia, and beyond.
- Approximately 90% of wheat varieties are susceptible to Ug99.
- In 2005, the famous scientist, Dr. Norman Borlaug established the Borlaug Global Rust Initiative aid the fight against Ug99.

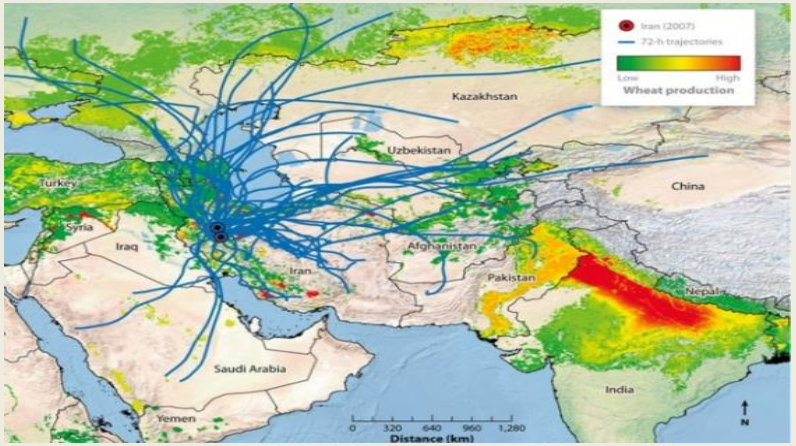


Photo Credit: Sing, RP e al, 2011

Ug99 Wheat Rust

Photo Credit: Petr Kosina/CIMMYT

Background Information Overview

Stem Rust Resistance Genes

- Over 50 stem rust resistance genes exist in wheat and its wild relatives.
- Most resistant genes are pathogen strain specific. However, Sr2 is strain-nonspecific and provides “durable resistance.” Sr2 slows rusting, which might prevent significant yield loss during severe epidemics. (USDA)
- Several stem rust resistance genes, including Sr2 and Sr35, are effective against Ug99. Two genes—Sr24 and Sr36—showed resistance to initial Ug99 strains but not the more recent, virulent strains.

Background Information Overview

Molecular Markers and Disease Resistance

- Conventional approaches for screening for stem rust resistance genes are not ideal because they require plants to be infected with the pathogen to identify resistance genes.
- DNA markers can be used to identify disease resistance genes.
- Technology that combines identification of molecular markers with breeding – “molecular breeding” – has enabled simultaneous introduction of genes into plants.

Research Methodology

- **Plant samples collection:** Seeds from 117 wheat varieties were obtained from the Pakistan National Agricultural Research Center. Seeds from each genotype were sown and leaf tissue from the plants was harvested 2-4 weeks after growth.
- **Genomic DNA Extraction:** Genomic DNA was extracted from leaf samples using chemical (cetyltrimethylammonium bromide) and physical (i.e., mortar and pestle) extraction. The quantity of DNA extracted was based on comparison with the band strength of the molecular weight standard.
- **Polymerase Chain Reaction Analysis:** Using standard polymerase chain reaction and gel electrophoresis, eighteen DNA markers were used to screen the plant samples for their presence or absence of stem rust resistance genes. These markers corresponded to (*Sr2*, *Sr6*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr26*).

Risk Analysis in this Research Article

While risk analysis is an important part of science, few scientific publications include in-depth descriptions of how the authors assessed and managed risk.

Today your task is to perform a risk analysis based on this research article.

To begin, answer the following question:

Based on your current knowledge of the experimental procedures or research purpose, what risks might be important to consider in designing, carrying out, or communicating this research?

Risk Identification

Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties

Questions
What, if any, are the potential biosafety and biosecurity risks to the environment or other laboratory plants from this research?
Could the research (pathogen strains or results) be used to deliberately cause harm? To wheat crops? To the environment?
What, if any, are the potential risks of the conventional approach for screening resistance genes?

Risk Assessment

Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties

Question
What, if any, risks were reduced by screening for resistance genes using molecular markers rather than the conventional approach?
To what degree is this research on highly pathogenic fungus, such as stem rust, a biosecurity or dual use hazard?
What are the resources, expertise, training, and tools that could be useful in assessing the risks identified for this research project?

Risk Management

Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties

Question
What relevant international, national, or institutional regulations, laws, and best practices exist to help minimize likelihood of the identified risks from this stem rust research?
What, if any, are the specialized competencies, skills, and training needed to carry out this research project, including successfully and safely obtaining, growing, and analyzing the specimen?
How much could the use of molecular markers reduce the biosecurity risks of conventional methods?
What approaches could be used to minimize the identified risks of an accidental release of laboratory grown plants into the environment?

Research Results and Conclusions

Results

- The stem rust resistance genes – Sr22, Sr24, Sr25, and Sr26 – were not present in the Pakistani wheat strains tested.
- The Sr2 gene was observed in the majority of wheat strains tested (variation was observed with different DNA markers used for Sr2 [range 9-79%]).
- Sr31 was observed in 35% of wheat strains, Sr6 was observed in 11% of wheat strains, and Sr38 was observed in 9% of wheat strains.

Conclusions

- The authors concluded that very few stem rust resistance genes are found in many of the wheat strains commonly found in Pakistan. They recommend conducting molecular breeding to create wheat strains that have the missing resistance genes.
- The authors also highlighted the need for “more reliable and efficient markers” to identify disease resistance genes.

Risk Communication

Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties

Question
What are the risks that should be communicated during this research? To whom?
How would you communicate the risks and risk management steps to an institutional ethics or environmental safety committee, funding organization, and the public?
Does doing research on a potentially harmful material or pathogen, such as wheat stem rust, change the communication strategy? If so, in what way?
Under what circumstances would researchers have an ethical or public safety obligation to share their findings with the public?
Could the identified risks outweigh the benefits of communicating the research results? If so, what could researchers do to minimize the possible risks of communicating the results?

Final Exercise: Risk in Your Own Research

Perform a risk analysis of your own research. Choose one past, ongoing, or future research project to analyze:

1. Identification: What are the primary risks you face in your research? Think about the risks to you and other researchers and technicians in the field, clinic, and/or lab, the general public, the environment and economy, your institution, and human and animal subjects.
2. Assessment: What are the consequences of the identified risks if they occur? How likely are they to occur? Based on your assessment of the potential consequences, are there are there any risks that could harm people, animals, crops, or the economy? What resources, capabilities, and skills are needed to mitigate these risks?
3. Management: What strategies could you use or resources you could refer to minimize or mitigate these risks? (These strategies should not decrease the quality of the research.) For ideas of possible strategies and resources, consider those discussed in this practical exercise and from your own experiences. Are there any risks associated with your research that cannot be adequately mitigated?
4. Communication: What risks, if any, are associated with communicating your research during the design or conduct of the research? What risks, if any, are associated with communicating the research results at scientific conferences and in publications? What strategies could you use to mitigate the risks? Are there any stakeholders with whom you must share or should share the risks of your research? Your findings?

Example Risk Analysis Framework

Communicate

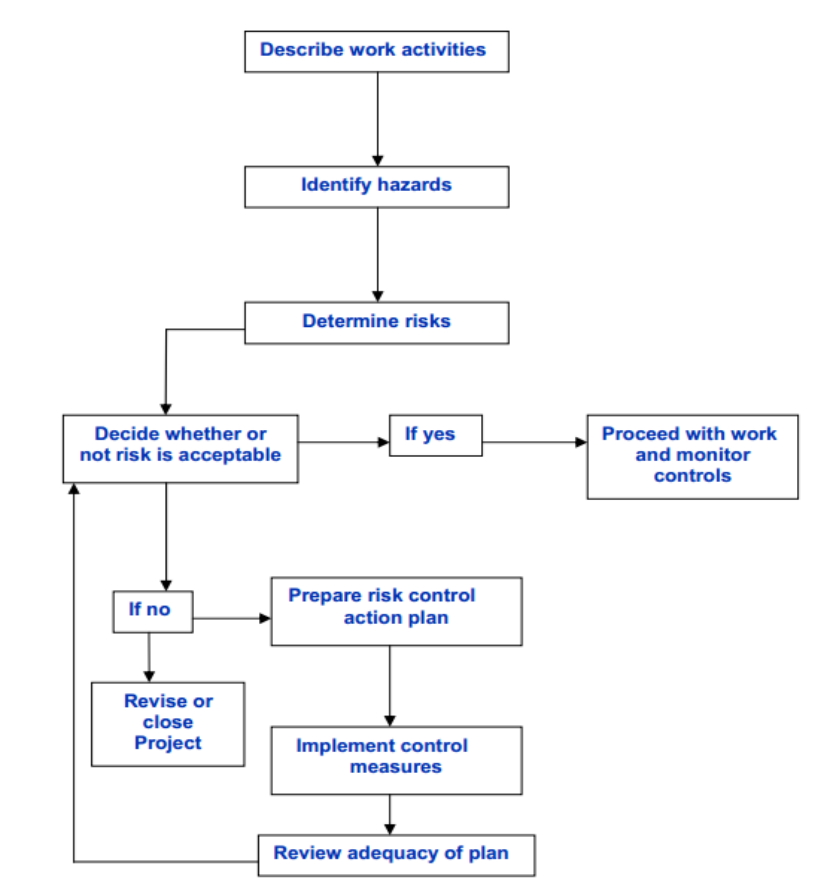


Figure 1 — Risk assessment strategy

"Laboratory risk management." CWA 15793: 2011

Reference List

Background Information and Data

Ejaz, M, et al. “Genetic Variation for Markers Linked to Stem Rust Resistance Genes in Pakistani Wheat Varieties.” Crop Science. 2012; 52: 2638-2648.

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Diagrams and Photos

Singh RP, et al. *The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production.* Annu. Rev. Phytopathol. 2011. 49, 465– 481 (2011).

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European Committee for Standardization (CEN). CEN Workshop Agreement: CWA 15793. “Laboratory biorisk management.” Ref. No: CWA 15793:2011 D/E/F. September 2011: 17. Available at: ftp://ftp.cenorm.be/CEN/Sectors/TCandWorkshops/Workshops/CWA15793_September2011.pdf.