

# **TOWARD THE DEVELOPMENT OF SOYBEAN VARIETIES RESISTANT TO RUST DISEASE**

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**NAOKI YAMANAKA** holds a Ph.D. degree in Agriculture from Chiba University (Japan) and is a Senior Researcher of JIRCAS. His expertise is in the area of genetics and breeding in plant. After working at Chiba University as a JSPS Research Fellow, he has worked at JIRCAS. In his 15-years' career as a researcher, he has studied mostly on soybean. Currently, he is in-charge of a research subject of the Pest and Disease Control Project in JIRCAS.



# ABSTRACT

Soybean is highly important for Japan as it is widely used in producing traditional soy foods as well as soybean oil. However, the domestic soybean demand in Japan is mostly met by imports from other countries. Therefore, it is important for us to ensure stable soybean production in the countries which supply large amount of soybean to the world market. South American countries such as Brazil, Argentina, and Paraguay produce more than half of soybean in the world market. But in the tropical and subtropical regions of these countries, a soybean disease, Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is one of the most serious threats to soybean growers together with drought. Thus, JIRCAS has been carrying out collaborative researches on the development of ASR-resistant cultivars with our partner institutions in South America for more than 10 years.

Firstly, we have developed a reliable method of evaluating ASR resistance in soybean and surveyed geographical and annual variations of ASR pathogen in South America (Yamanaka et al. 2010). Our results reveal that virulence of South American ASR pathogen is highly variable and strong (Akamatsu et al. 2013). Therefore, a resistant cultivar carrying suitable ASR resistance cannot be expected by simply introducing single known major ASR resistance gene. Secondly, we have developed soybean breeding materials and tools which are useful in South America. Specifically, we have 1) identified resistance genes/alleles in the ASR-resistant soybean genotypes whose resistance genes/alleles were unknown (Hossain et al. 2015; Yamanaka et al. 2015a; Yamanaka et al. 2016); 2) explored DNA markers for newly and previously identified resistance loci, and 3) developed and evaluated soybean breeding materials carrying multiple ASR resistance genes (Lemos et al. 2011).

Through these works, it appears that introducing multiple resistance genes into single soybean genotype brings high ASR resistance. This high resistance also acts synergistically in gene pyramided soybean lines, when they are inoculated with the *P. pachyrhizi* races which are virulent to each of the pyramided genes (Yamanaka et al. 2015b). JIRCAS and our partner institutions in South America have carried out some marker-assisted breeding programs to introduce this high ASR resistance in South American soybean cultivars by utilizing the gene pyramided lines.

## KEYWORDS

Asian soybean rust; Breeding; Gene pyramiding; Marker-assisted selection (MAS); Resistant variety

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**JIRCAS**

**Toward the Development of Soybean Varieties Resistant to Rust Disease**

JIRCAS Soybean Research in South America

Naoki Yamanaka, Ph.D.  
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Japan International Research Center for Agricultural Sciences



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**Contents**

1. Why soybean in South America is important?
2. Our research target: Asian soybean rust disease
3. Pathogenic characteristics of rust pathogen in S.A.
4. Breeding program for rust-resistant soybean in S.A.



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**Why soybean in S.A. is important?**




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**Soybean is an important food resource for Japanese**

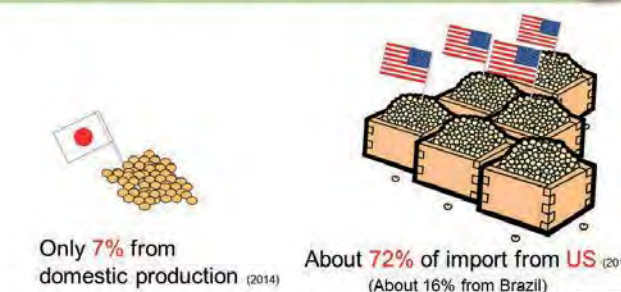


Soybean is also used as feed, cooking oil (65% in JP), and food additives (emulsifier)



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**Our soybean consumption is depending on import**




Only **7%** from domestic production (2014)

About **72%** of import from **US** (2015)  
(About 16% from Brazil)

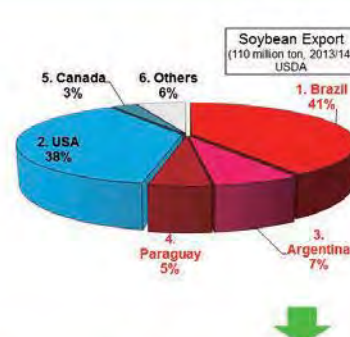
MAFF, MOF

A jump in international soybean price influences our living cost directly!




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**Soybean production in S.A.**




3 countries in South America export more than 58 million ton (53% of world market, and 18 times of Japanese import)

**Stable soybean production in S.A. is important for Japan, too**



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## What is the problem of soybean production in S.A.?


Our research target:  
**Asian soybean rust disease**

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## Asian soybean rust (Pathogen: *Phakopsora pachyrhizi*)





**Rust was 1stly detected in 2001 and got to be the most serious disease in S.A.**

Economical damage by rust is estimated as 24 billion USD in Brazil from 2002 to 2014 (Yield loss and control cost)

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## Development of varieties resistant to rust

### Introducing resistant varieties


Low cost  
Environmentally friendly




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## Genetic resistance to soybean rust






**Resistant** **Susceptible**



7 kinds of resistance genes named as "*Rpp*" have already been identified

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## Characteristics of soybean rust in S.A.

At least 7 resistance genes are available for breeding


Which gene is effective?  
How much and how long it will be effective?


We decide breeding material and strategy based on the pathogenicity of pathogen



**Research Subject (1) with the partner institutions in S.A.**

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## Pathogenic characteristics of rust pathogen in S.A.

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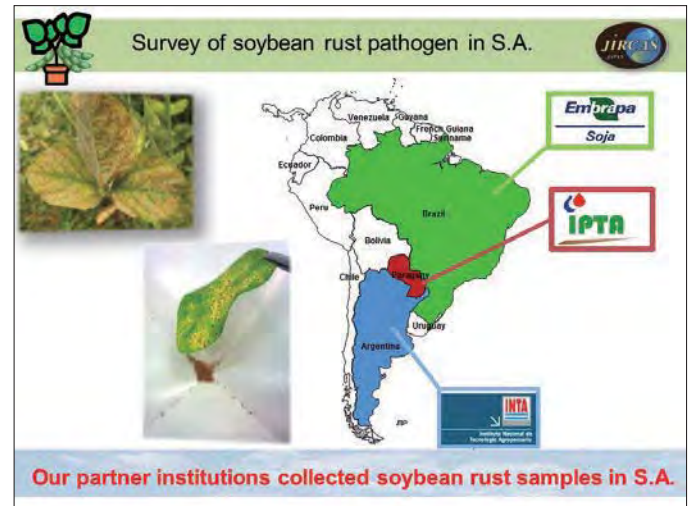
**Differential varieties to check pathogenicity**

No.	Name	Alternative name	R gene	Origin
RDV1	PI 200492	Komata	<i>Rpp1</i>	Japan
RDV2	PI 587886	Bai Dou	<i>Rpp1</i>	China
RDV3	PI 230970	No. 3	<i>Rpp2</i>	Japan
RDV4	PI 462312	Ankur	<i>Rpp3</i>	India
RDV5	PI 416764	Akasaya	<i>Rpp3</i>	Japan
RDV6	PI 459025	Bing Nan	<i>Rpp4</i>	China
RDV7	PI 200526	Shiranui	<i>Rpp5</i>	Japan
RDV8	PI 567102B	MARIF 2767	<i>Rpp6</i>	Indonesia
RDV9	PI 587880A	Huang Dou	<i>Rpp1-b</i>	China
RDV10	PI 594767A	Zhao Ping Hei Dou	<i>Rpp1-b</i>	China
RDV11	BRS154	-	(None)	Brazil
RDV12	No6-12-1	-	<i>Rpp2, 4, 5</i>	Japan

Yamanaka et al. (2016)  
Lab manual v.22

**Differential varieties carry different resistance genes to investigate pathogenicity of soybean rust pathogen**

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**Pathogenicity of rust pathogen in S.A.**

**Soybean rust pathogen**

Differential variety	Rpp gene	Pathogenicity (R: Resistant, M: Intermediate, S: Susceptible, -: no data)
RDV1	<i>Rpp1</i>	...
RDV2	<i>Rpp1</i>	...
RDV3	<i>Rpp2</i>	...
RDV4	<i>Rpp3</i>	...
RDV5	<i>Rpp3</i>	...
RDV6	<i>Rpp4</i>	...
RDV7	<i>Rpp5</i>	...
RDV8	<i>Rpp6</i>	...
RDV9	<i>Rpp1-b</i>	...
RDV10	<i>Rpp1-b</i>	...
RDV11 (S)		...
RDV12 3Rpp		...

Legend: R : Resistant, M : Intermediate, S : Susceptible, - : no data

Akamatsu et al. (2016)  
JARQ Accepted

**Highly virulent, large variation, No *Rpp* gene is resistant to all !  
Introducing single *Rpp* gene into cultivars isn't always function**

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**Strong enemy: Rust pathogen in S.A.  
How do we defeat it?  
R gene pyramiding**

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**Effect of resistance gene pyramiding**

Resistance gene pyramiding or *Rpp*-pyramiding mean "introducing multiple resistance genes (*Rpp* genes) into a single soybean plant"

2 kinds of effects are expected in the *Rpp*-pyramiding

- 1) Resistance to larger numbers of rust races and
- 2) Stronger resistance by synergy effect

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**Effect of resistance gene pyramiding**

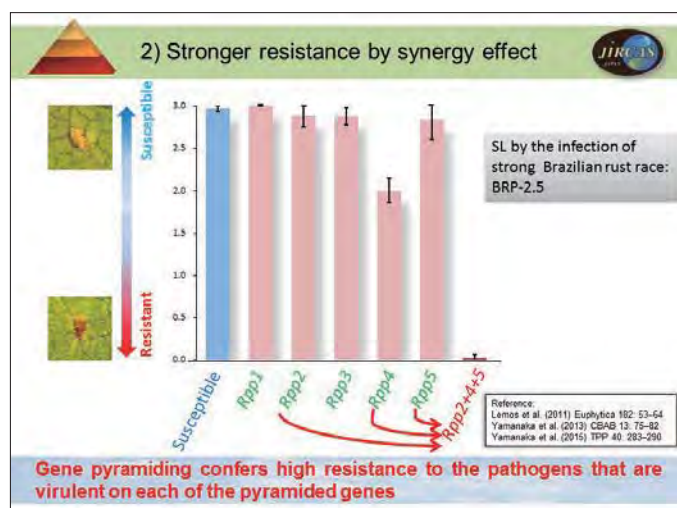
2 kinds of effects are expected in the *Rpp*-pyramided lines.

- 1) Resistance to larger numbers of rust races and
- 2) Stronger resistance by synergy effect

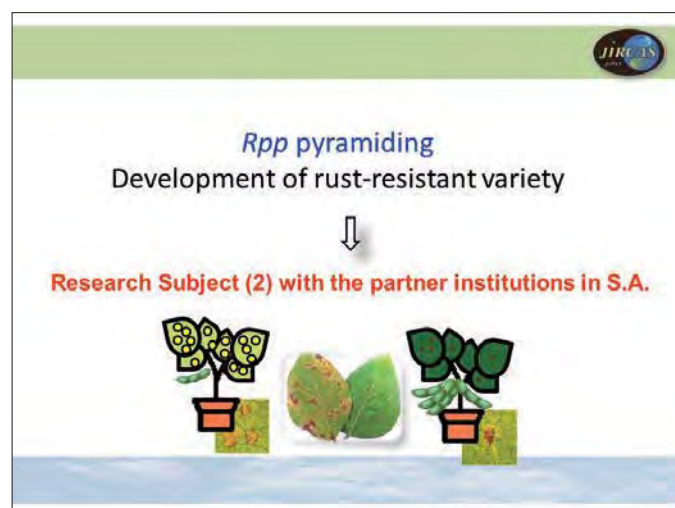
1. Rust pathogen in S.A. has large pathogenic variation → Effect 1)  
2. Rust pathogen in S.A. is highly virulent → Effect 1), 2)  
3. No *Rpp*-gene is resistant to all S.A. races → Effect 2)

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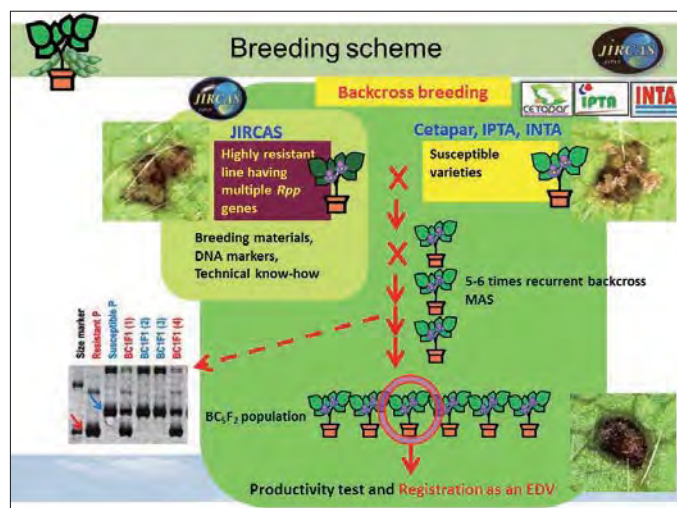




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### New rust-resistant variety, JFNC 1 in Paraguay

Senave: Servicio Nacional de Calidad y Sanidad Vegetal y de Semillas (SENAVE)  
División General de Semillas (DISE)  
Departamento de Protección y Uso de Variedades (DPUV)

E. Comparación entre descriptores del cultivar presentado para Protección, con los descriptores del cultivar más parecido.

CARACTERÍSTICA	JFNC 1 CULTIVAR PRESENTADO	AURORA CULTIVAR MÁS PARECIDO
Color del hipocotilo (1)(8)	Purpura (1)	Purpura (1)
Tipo de crecimiento (4)(13)	Determinado (1)(8)(9)(10)	Determinado (1)(8)(9)(10)
Color de la flor (3)(8)	Purpura (1)	Purpura (1)
Color de la pubescencia del tallo principal (1)(11)(12)	Gris (1)(11)	Gris (1)(11)
Forma del folio lateral (8)(9)	Folículo oval - redondeado (8)(9)	Folículo oval - redondeado (8)(9)
Color de fondo del leguminoso (incluyendo el tallo principal) (3)(8)	Amarillo (8)(9)	Amarillo (8)(9)
Color de la pubescencia de la vena principal (1)(11)	Gris (1)(11)	Gris (1)(11)
Color del tallo (1)(11)(12)	Amarillo (8)(9)	Marrón claro (8)(9)
Ciclo total (1)(13)	145 días (1)	130 días (1)
Altura de planta (1)(13)	85 cm	85 cm
Peso de 100 semillas (10)(11)	18.5 g	18.5 g
Tiempo de secado (10)(11)(12)	21.3	22.9
Tiempo de germinación (10)(11)(12)	40.7	39.8
Características de la semilla (1)(11)(12)	Resistente (1)(11)	Resistente (1)(11)
México (VASC) (1)(11)(12)	San Gato (1)(11)(12)	Mediano Resistente (1)(11)
Reja de la soja (1)(11)(12)	Resistente (1)(11)	Susceptible (1)(11)

JIRCAS and Cetapar have developed new variety jointly

JFNC 1 has similar characteristics to the original variety, Aurora except for high soybean rust resistance

Several more varieties will be developed over the next few years!

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## International Soybean Rust Research Network

Country	Institution	Name				
Japan	JIRCAS	N Yamanaka (Researcher)	M Kato (Project leader)			
		M Morishita (Assistant)	M Hasegawa (Assistant)	Y Muraki (Assistant)		
	Tsukuba Univ.	Y Yamaoka (Professor)	Y Ishiga (Assistant Prof.)			
	Kureha Ltd.	H Tateishi (Researcher)				
Paraguay	Nikkel-Cetapar	M Uno (Technician)				
	IPTA-CICM	C Dujak (Technician)	A Morel (Breeder)	R Schloz (Researcher)	G Morel (Technician)	
Argentina	INTA	M I Heck (Technician)	AD de Lucia (Breeder)	AJG Ivanovich (Researcher)		
	Brazil	Embrapa-Soja	RM Soares (Researcher)			
Uruguay	INIA	S Stewart (Researcher)	S Ceretta (Breeder)			
	Mexico	INIFAP	HMC Escobar (Researcher)	JCG Rodriguez (Researcher)	NM Moreno (Researcher)	MAC Cruz (Researcher)
Bangladesh	BSMRAU	MM Hossain (Associate Prof.)				
	Tanzania	IITA	HM Muruthi (Research Fellow)			

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### ご清聴ありがとうございました

Thank you for your attention

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**Chair Nakashima**

Our last speaker for this session is Dr. Naoki Yamanaka. He is Senior Researcher of JIRCAS and he studied on the soybean for about 15 years in JIRCAS. Today, he will present on 'Toward the Development of Soybean Varieties Resistant to Rust Disease.' Dr. Yamanaka, please go ahead.

**Dr. Naoki Yamanaka**

Thank you Dr. Nakashima for the kind introduction. Good afternoon, ladies and gentlemen. I am Naoki Yamanaka, Senior Researcher of JIRCAS. It is my great pleasure to introduce our research in this symposium. The title of my presentation today is 'Toward the Development of Soybean Varieties Resistant to Rust Disease.' And this is one of JIRCAS soybean research activities in South America.

In my presentation, at first I would like to talk why soybean production in South America is important. In second, our research target: Asian soybean rust disease. And third, pathogenic characteristics of rust pathogen in South America. Finally, I would like to introduce our breeding program for rust-resistant soybean in South America.

First of all, let me explain why soybean in South America is important. As you may know, soybean is an important food resource for us, Japanese. Soybean is used for various Japanese foods, Tofu, Natto, Sho-yu, Miso and so on. Soybean is also used for feed for livestock, cooking oil material, and sometimes we use it for food additives such as emulsifier.

Soybean is very important; however, our soybean consumption is depending on import. We can produce only 7% of our consumption. So, we have to import, and about 72% of imported soybean comes from US, the 1st producer in the world. So, a jump in international soybean price influences our living cost directly. This is the problem.

How do we solve this problem? One solution is soybean production in South America. This slide shows us major soybean exporters in the world market. Three South American countries; Brazil, Argentina, and Paraguay export more than 58 million ton of soybean, and this is equivalent for 53% of world market and 18 times of Japanese import. I mean huge amount. So, soybean production in South America gets to be more stable or increase and soybean price gets to be cheaper, we can import more soybean from South America. So, stable soybean production in South America is important, not only for their country, but also for Japan.

Then, what is the problem of soybean production in South America? One serious problem is our research target: Asian soybean rust disease. Asian soybean rust disease is caused by the pathogen *Phakopsora pachyrhizi*. Soybean rust was firstly detected in South America in 2001 and got to be the most serious soybean disease there. This picture shows the soybean field seriously damaged by this disease. You can see the soybean plants lost their leaves before maturation. So, farmers cannot expect a yield in this situation. According to South America Anti-Rust Consortium, economical damage by this disease is estimated as 24 billion USD only in Brazil from 2002 to 2014. So, soybean rust is a very serious problem. So, we decided to study on development of varieties resistant to rust to control this disease, because introducing resistant varieties has merits, low cost and environmental friendly.

For genetic improvement, genetic resistance to pathogen is necessary. Fortunately, for soybean rust, seven kinds of resistance genes named as *Rpp* have already been identified. If soybean plants carry the resistance gene and the pathogen is avirulent to that resistance gene, soybean plants show resistant reaction like this picture of immunity, so soybean plant can keep green healthy leaves. Contrastingly, if soybean plant doesn't have any resistance genes, actually most of cultivars, or even the resistant variety, if the pathogen is virulent to that resistance gene, soybean plant shows susceptible reaction to produce lots of spores like this picture, the leaves of plant yellow and fall.

As I mentioned, at least seven kinds of resistance genes are available for breeding, but which gene is effective and how much and how long it will be effective. This is important point because we decide breeding material and strategy based on the pathogenicity of pathogen. This is important work. So, we carried out this important work with our partner institutions in South America.

Next, I would like to show the results of this collaboration, pathogenic characteristics of rust pathogen in South America. To determine pathogenicity, pathogenic characteristic of pathogen, differential varieties carrying different kind of resistance genes are needed. This list shows our differential variety set consisting

of 12 varieties, 10 of 12 varieties carrying single *Rpp* gene differently and one variety has no resistance gene. This is a susceptible check. And one line carrying three genes. About this line, I am going to explain later.

Our partner institutions collected soybean rust samples in their country and then they inoculate these samples to the differential varieties and they evaluate the reaction on the differentials.

This slide shows the results, pathogenicity of rust pathogen in South America. These are the soybean rust pathogenic samples collected in Argentina, Brazil, and Paraguay. Actually, they have collected more than 140 samples in 7 years, but here I show only partial data. And these are differential varieties.

In this table, Red R shows resistant reaction was observed. So that resistance gene was effective to that pathogen. Blue S means susceptible reaction was observed. So, that gene was ineffective. In other words, the pathogen was virulent to that resistance gene. Yellow IM means intermediate reaction was observed. This phenotype is phenotype between resistant and susceptible. Resistance genes show the resistance little and pathogen showed virulent partially.

What we get to know? The first, as you can see, many S reactions were observed. So, South American pathogen is highly virulent. In second, each pathogenic sample shows different pattern against different varieties, so South American pathogen has large variation. And third, this is most important point, some resistance genes tend to show resistant reaction to the pathogen with high frequency, but there's no resistance gene resistant to all tested samples. So, if we introduce the single resistance gene into cultivars, we cannot expect stable resistance for that variety.

Now, we know that South American pathogen is very strong, how do we defeat it? Our choice is gene pyramiding.

Please let me explain about gene pyramiding. Resistance gene pyramiding or *Rpp*-pyramiding, in case of soybean rust, means introducing multiple resistance genes, in case of soybean rust, *Rpp* genes, into a single soybean plant. Two kinds of effects are expected in the *Rpp*-pyramiding. First, resistance to larger numbers of rust races. In second, stronger resistance by synergy effect. Please remember the characteristics of the South American rust pathogen. South American rust pathogen has large pathogenic variation and highly virulent, and there is no resistance gene resistant to all South American races. For the first character of the pathogen, pyramiding effect 1 is useful. For the second character of the pathogen, both effects are useful. And for the third character of the pathogen, pyramiding effect 2 can defeat it.

Okay, next, let me explain a little bit more about the second effect of pyramiding, stronger resistance by synergy effect. This graph shows the sporulation level by the infection of strong Brazilian rust race BRP-2.5 in susceptible variety, resistance varieties carrying single gene, and *Rpp*-pyramid line. Susceptible variety and resistance varieties carrying single gene showed moderate level of sporulation or maximum level of sporulation to produce lot of spores to be susceptible like this picture. Contrastingly, the *Rpp*-pyramided line carrying three genes showed almost no sporulation to be highly susceptible phenotype. This is one typical example of the pyramiding effect. Gene pyramiding confers high resistance to the pathogens that pathogens are virulent on each of the pyramided genes. So, now we know that resistance *Rpp* gene-pyramiding should be useful for the development of resistance soybean rust variety. So, we decided to use *Rpp* gene pyramiding for breeding. And we carried out this work with our partner institution in South America.

This slide shows our breeding scheme carried out with our partner institutions; Cetapar, IPTA in Paraguay and INTA in Argentina. We, JIRCAS, develop and provide highly resistant line carrying multiple resistance genes as breeding materials together with DNA markers and technical know-how to use them. Our partner institutions, they have their own varieties. These varieties are nice to show good adaptability or productivity in their countries, however, unfortunately, susceptible to rust. So, we carry out backcross breeding by using our *Rpp*-pyramided line as donor parent and their soybean variety as recurrent parent. After 5 or 6 times recurrent backcrossing and marker-assisted selection, we choose several lines. And after productivity test in multi-locations, we choose one single line as candidate variety that shows the strong resistance by gene pyramiding but similar agronomical characteristics as the recurrent parent, and we register it as an **Essentially-Derived Variety**.

JIRCAS and Nikkei-Cetapar started this breeding program 7 years ago and we developed a new rust-resistant variety, JFNC 1, JIRCAS and Foundation Nikkei-Cetapar 1 in Paraguay. We applied variety registration just



last month, so this variety has not been released yet in the market. But we have already confirmed JFNC 1 has similar characteristic to the original variety, Aurora except for high soybean rust resistance. This picture shows the JFNC 1 and Aurora are growing in soybean rust-occurring field in Paraguay. JFNC 1 keeps more green leaves than Aurora. Under the rust disease controlled condition by fungicide, these two varieties show similar agronomical characteristics each other. Except for JFNC 1, several more varieties are under development. So, over the next few years, we will complete the development varieties and release them in near future.

In the end of my presentation, I'd like to thank my colleagues in Japan and my staffs in JIRCAS and especially my friends in our partner institutions in South America.

In this presentation, I could introduce part of our collaboration with Argentina, Brazil, and Paraguay, but our International Soybean Research Network is expanding to other surround regions and other countries, for example, Uruguay, Mexico, Bangladesh, and Tanzania. So, by developing good rust-resistant variety in these countries, I hope soybean rust disease gets to be a minor disease or past disease in this country in some day. That's all of my presentation. Thank you for your attention.

#### **Chair Nakashima**

Thank you very much for the nice presentation, Dr. Yamanaka. He has showed that JIRCAS and the partner institutions in South America have succeeded to introduce the high rust resistance in South American soybean cultivars by pyramiding resistance genes. So, it's open for the discussion. Are there any questions or comments? Okay.

#### **Male Questioner**

At first, I appreciate your considerable activities and work. As you successfully introduced gene resistance to rust and soybean, have you ever illustrated the effects of that gene in other component like yield and yield component in soybeans, its effect? Effect of that gene and yield component on other traits?

#### **Dr. Naoki Yamanaka**

Do you mean under the soybean rust occurring condition or without soybean rust disease occurring condition?

#### **Male Questioner**

In two conditions comparison. You introduced the gene successfully. Have you elucidated the effect of that gene on other traits like yield component?

#### **Dr. Naoki Yamanaka**

Okay. These resistance genes some of them have already isolated and it is NBS-LRR gene, the R gene, and it does not influence the other characters, only the resistance. We carefully checked the developed line if there is any other effect by these genes, but until now we have not observed. So, basically, that gene influences on the resistance only.

#### **Chair Nakashima**

Okay? Are there any questions or comments?

#### **Male Questioner**

Thank you for your presentation. Is there any update on the biochemical basis of your gene?

#### **Dr. Naoki Yamanaka**

Sorry, we haven't done any work regarding that.

#### **Chair Nakashima**

Are there any questions or comments? Can I ask one question? How is the plan for the release of the practical variety in Latin America? When can you release the practical variety?

#### **Dr. Naoki Yamanaka**

As I introduced the first variety has just applied registration. So, we have to wait for the result first. I think not so long time it takes, but after that, we are planning to ask the farmers to cultivate this variety.

**Chair Nakashima**

Thank you. I hope this will be used in Latin America. Okay. It's almost time, so thank you very much, Dr. Yamanaka. It's now time to close this session. I think we were able to share valuable information on some interesting research topics related to pulse research in India and some interesting genes of *Vigna* and breeding research of soybean against disease. I would like to close this session by thanking all the presenters for their contributions. Please give a big hand to the speakers. Thank you very much.



Questioner



Questioner