'Đ9', a high-yielding and early maturing soybean cultivar resistant to soybean rust isolates from Vietnam

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Abstract: *A new soybean cultivar, Đ9, has the advantages of its parental lines: the Rpp2 resistance gene from the male parent (ĐT2000), and early maturation from the female parent (TL7). Đ9 displayed high resistance to soybean rust isolates from Vietnam, short-medium time to maturity, and high grain yield. This cultivar has been released for commercial production in northern Vietnam.*

Keywords: *Glycine max (L.) Merill, Asian soybean rust, Rpp resistance genes, pedigree method, SSR markers*

INTRODUCTION

The soybean (*Glycine max L.*) is an important crop worldwide due to the high protein and lipid content in soybean seeds. In Vietnam, the demand for soybeans for production of human food and animal feeds has increased rapidly in recent years. The soybean market in Vietnam in 2019 was over 3 million tons, valued at around 2.5 – 3 billion US dollars. More than 90% of the soybeans consumed in Vietnam are imported, mainly from Brazil, the USA, and Canada. The low contribution of domestic production (<10%) is the result of many barriers, including a lack of high yield, high plasticity varieties, as most farms are relatively small and unsuited to mechanization. These limitations underlie significant additions to production cost (10 %–12% according to data published in 2019 by the General Statistic Office of Vietnam at https://www.gso.gov.vn).

Soybean growing season varies across Vietnam, depending on region (Supplementary Table 1). Soybean production has the potential to expand into 4 million hectares of rice land in the Mekong and Red River deltas. The two most important requirements for a soybean cultivar in these deltas are early maturity and high plasticity in response to biotic and abiotic stresses. Among pests and diseases that affect soybeans, soybean rust caused by the fungus *Phakopsora pachyrhizi* is a major concern because of its negative impacts on soybean growth and yield (Araújo and Vello 2010, Santos et al. 2018). Introducing resistance gene(s) into soybean cultivars has been used as one strategy to control rust disease (Yamanaka et al. 2013, Kashiwa et al. 2020). To date, seven loci carrying Asian soybean rust (ASR) resistance genes have been reported: *Rpp1* identified in PI 200492 by using SSR marker Sat_046, Sct_187 on chromosome 18 (Hyten et al. 2007); *Rpp1-b* (PI 594538A, marker: Sat_372, Sat_064 on chromosome 18, the same loci as *Rpp1*) (Chakraborty et al. 2009) , *Rpp2* (PI 230970, marker:

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Table 1. Agronomic traits of the Ð9 cultivar and its two parental lines

Trait	Winter 2015			Spring 2016		
	Ð9	ÐT2000	TL7	Ð9	ÐT2000	TL7
Plant height (cm)	45.5	55.0	44.5	65.3	66.5	53.0
Days to maturity (days)	89	110	83	100	130	90
Number of branches	2.4	2.5	2.2	3.5	3.5	3.0
Leaf color	Light green	Green	Light green	Light green	Green	Light green
Flower color	Purple	Purple	Purple	Purple	Purple	Purple
Pod color	Yellow	Yellow	Yellow gray	Yellow gray	Yellow	Yellow
Grain-coat color	Light yellow	Yellow	Light yellow	Light yellow	Yellow	Light yellow
Grain hilum color	Brown	Brown	Brown	Brown	Brown	Brown
Pods per plant	38.6	48.4	35.5	42.0	53.2	41.0
Number of grains pod^{-1}	2.3	2.4	2.2	2.5	2.5	2.6
100-grain weight	19.4	18.3	20.1	17.5	16.7	18.5
Protein content (%)	-	-	-	37.0	40.7	38.6
Lipid content (%)	-	-	-	18.0	20.6	18.4
Infected by *R. bataticola* fungi * (%)	5-6	7-8	7-8	5-6	5-6	7-8
Infected by *E. diffusa* fungi ** (1-5)	1-2	2-3	1-2	1-2	2-3	1-2
Infected by *P. manshurica* fungi *** (1-9)	1-3	1-3	1-3	1-3	3-5	1-3

*: Dry root rot disease score was calculated as the percentage of plants infected by *Rhizoctonia bataticola* fungi.
**: Soybean powdery mildew disease score was classified by percentage of infected plants (1: <5%, 2: 6-25%, 3: 26-50%, 4: 51-75%, 5: >75%).
***: Soybean downy mildew disease score was classified by percentage of infected leaf area (1: <1%, 3: 1-5%, 5: 6-25%, 7: 26-50%, 9: >50%). Measurements were taken in winter 2015 and spring 2016 at the Field Crops Research Institute, Hai Duong province.

Satt215, Satt620, Sat_255 on chromosome 16) (Silva et al. 2008), *Rpp3* (PI 462312, marker: Satt460, Sat_251, Sat_263 on chromosome 6) (Hyten et al. 2009), *Rpp4* (PI 459025B, marker: Sat_191, Satt288 on chromosome 18) (Silva et al. 2008), *Rpp5* (PI 200456, marker: Sat_275, Sat_280 on chromosome 3) (Garcia et al. 2008), *Rpp6* (PI 567102B, marker: Satt324, Satt394 on chromosome 18) (Li et al. 2012), and *Rpp7* (PI 605823, marker: GSM0461/ GSM0468 on chromosome 19) (Childs et al. 2018). A set of these PI accessions are commonly used as positive controls to distinguish *Rpp* resistance genes in cultivars, or as *Rpp* donors in breeding programs for ASR resistant soybean varieties (Yamanaka et al. 2013, Aoyagi et al. 2020).

In the current breeding project, we conducted a large screen for *Rpp* resistance genes and introduced a suitable gene (*Rpp2*) into cultivars by conventional crossing, with the aid of pedigree information and SSR marker-assisted selection. We successfully produced a new soybean cultivar named Ð9, which displays medium length time-to-maturity, high yield, and resistance to soybean rust disease.

BREEDING METHODS

The Ð9 cultivar was developed from a conventional crossing between two commercial cultivars, ÐT2000 and TL7. The male parental line (ÐT2000) has good yield and resistance to soybean rust (it carries Rpp2 and Rpp4 resistance genes) (Pham et al. 2010), while TL7 provides the advantage of short maturation time.

Parental line selections

Parental lines were selected from among 65 cultivars and pure lines provided by three research institutes (the Field Crops Research Institute, the Plant Resources Center, and the Vietnam National University of Agriculture) affiliated with the MARD. Parental lines were selected in winter 2012 based on the presence of *Rpp* genes and reactions to *P. pachyrhizi* in an inoculation experiment.

Simple sequence repeat (SSR) markers representative of the five *Rpp* genes were used to verify *Rpp* gene presence. Five accessions (PI 200492 (*Rpp1*, PI 230970 (*Rpp2*, PI 462312 (*Rpp3*, PI 459025B (*Rpp4*, and PI 200456 (*Rpp5*)) served as positive controls for Rpp resistance genes. These positive controls were originally collected, named, and characterized by the USDA Soybean Germplasm Collection, and were provided by the World Vegetable Research and Development

Center (AVRDC), Taiwan. These SSR markers were selected based on previous reports, and on the polymorphism displayed among their experimental cultivars. Specifically, the markers used were Sat_064 (*Rpp1*), Satt620 (*Rpp2*), Sat_263 (*Rpp3*), Satt288 (*Rpp4*), and Sat_275 (*Rpp5*) (Supplementary Table 2). The results of *Rpp* resistance gene screening are presented in Supplementary Table 3. Twenty-eight cultivars were identified that carried *Rpp* resistance gene(s).

By inoculation, cultivars were tested for their reactions to three isolates of *P. pachyrhizi* (IS-15, IS-17, and IS-28) from Vietnam. These respective isolates were collected in 2012 from Vinh Phuc (northern), Thanh Hoa (central), and Dong Thap (southern) provinces of Vietnam (Supplementary Table 4a). Isolates were purified by culture from single-spores and maintained on the susceptible cultivar ĐT12 in a quarantine net house at the Plant Protection Research Institute. Inocula were collected from typical Tan lesions on old ĐT12 leaves. A concentration of 5.10^4 spores per milliliter in deionized water was sprayed on both sides of young leaves at a dose of 0.5 mL dm^{-2} of leaf. The reactions of 65 cultivars to *P. pachyrhizi* were evaluated by lesion color (RB_ reddish brown represents resistance, TAN_ tan-color indicates susceptibility, and a mixed_appearance (MIX) represents presence of both RB and TAN phenotypes). RB lesion color was taken to indicate resistance (R), whereas TAN or MIX lesions were classified as susceptible (Maphosa et al. 2013). The results of inoculation assessment of 65 cultivars are reported in Supplementary Table 4b. Inoculation experiments were also performed on potential lines from the F_7 generation, as well as a national trial in 2016. In these experiments, reactions to *P. pachyrhizi* were evaluated based on lesion color and two other indicators: (1) scoring of area under the disease progress curve (AUDPC) (measured at 14, 21, and 28 days after inoculation), and (2) sporulation intensity, scored by recording the percentage of lesions that produced spores (0: no lesion produced spores, 1: 0–10%, 2: 10–25%, 3:

Table 2. Response of Đ9 and its parental lines to infection by two rust isolates in spring 2016

No	Cultivar	Reaction to soybean rust isolates					
		IS − 15			IS − 17		
		AUDPC*	Reaction type**	Sporulation intensity score***	AUDPC	Reaction type	Sporulation intensity score
1	Đ9	600	RB	3	580	RB	2
2	TL7	950	TAN	4	920	TAN	5
3	ĐT2000	690	RB	1	680	RB	1

*AUDPC (area under the disease progress curve) was measured at 14, 21, and 28 days after inoculation.
** Reaction type was determined by lesion color (RB_ reddish brown represents resistance, TAN_ tan-color indicates susceptibility, Mixed appearance (MIX) of both phenotypes (RB and TAN lesions)
*** Sporulation intensity scored by percentage of lesions producing spores (0: no lesions produce spores, 1: 0-10%, 2: 10-25%, 3: 25-50%, 4: 50-75%, 5: more than 75%)

Table 3. Grain yield of Đ9 and DT84 in the VCU experiments at six provinces during the period 2015-2016

Season	Cultivar	Experimental sites						Mean (year)
		Hanoi	Hai Duong	Thai Binh	Vinh Phuc	Nghe An	Thanh Hoa	
Winter 2015	Đ9	18.9	22.5	23.8	23.9	19.9	22.1	20.8
	DT84	16.6	21.1	19.2	19.2	18.2	19.2	18.5
Đ9 – DT84		2.3	1.4*	4.6*	4.7*	1.7	2.9*	2.3
CV%		8.2	2.0	5.7	5.9	7.8	2.3	
LSD (0.05)		3.1	1.0	2.6	2.8	3.4	1.0	
Spring 2016	Đ9	20.2	25.0	34.7	24.7	-	-	25.6
	DT84	18.7	18.9	29.4	20.4	-	-	21.2
Đ9 – DT84		1.5	6.1*	5.3	4.3*	-	-	4.4
CV%		6.6	5.0	7.6	6.2	-	-	
LSD (0.05)		3.1	2.7	5.5	3.4	-	-	
Winter 2016	Đ9	14.6	18.6	25.4	21.2	-	-	19.9
	DT84	15.3	16.7	23.7	18.6	-	-	18.6
Đ9 – DT84		-0.7	1.9	1.7	2.6*	-	-	1.3
CV%		8,8	6.0	8.1	2.3	-	-	
LSD (0.05)		3,1	2.5	4.6	1.0	-	-	

Unit: 100 kg ha^{-1}.
The Statistical tool for Agricultural Research (STAR) software, version 2.0.1 (2014), was used to conduct analysis of variance (ANOVA) at P≤0.05. LSD (0.05): least significant difference at P ≤ 0.05; *: significant at p ≤ 0.05. CV%: Coefficient of variation

25–50%, 4: 50–75%, 5: more than 75%) (Pham et al. 2010, Aoyagi et al. 2020).

Correlating *Rpp* gene presence with reaction results from the 65 cultivars indicated that *Rpp2* and *Rpp4* are the most effective resistance genes against Vietnamese soybean rust isolates (Supplementary Table 5). Ten cultivars were thus selected as parental lines, including five male parents carrying either *Rpp2* or *Rpp4*: ĐT2000 (*Rpp2 + Rpp5*), DT95 (*Rpp1 + Rpp 4*), Cao Bang U8325 (*Rpp1 + Rpp2*), ĐT92 (*Rpp2 + Rpp5*), Nhat Tien HLLS (*Rpp1 + Rpp2 + Rpp4*) (Supplementary Table 3), and five female parents: Đ8, TL7, M103, DT84, AK03. Female parents were selected for their desirable agronomic characteristics, including growth habits, plant structure, and high grain-yield. The ten selected parental lines were used to create 12 hybrids in the spring of 2013. These hybrids were used to generate 12 segregating populations for breeding purposes.

Pedigrees and breeding methods

A single plant selection method was used on generations F_2 to F_4. Twelve F_2 populations were grown separately. In each population, plant growth and morphology were first evaluated, followed by a screen assessing presence of *Rpp-resistant* genes. A total of 1.817 plants with good morphology were selected for *Rpp* resistance gene(s) screening, and 233 individuals in 12 F_2 populations with either *Rpp2* or *Rpp4* were identified and selected (Supplementary Table 6). Evaluations of 233 F_3 lines and 1.209 F_4 lines were based on easily observed characteristics, including plant growth, plant structure, short maturation time (<100 days), and yield. In the F_5 generation (715 lines), a mass selection method was applied to select 60 lines with good agronomic characteristics and a high level of uniformity. The 60 lines in the F_6 generation were evaluated for 11 agronomic traits, resulting in selection of 13 promising lines to establish F_7 generations (Supplementary Table 7). The lines in the F_7 generation were re-assessed for *Rpp* resistance gene(s) and for their reactions to soybean rust in an inoculation experiment (Supplementary Table 8). They were subsequently evaluated for agronomic performance (Supplementary Table 9). Eventually, the elite line VD1-2-1, which possessed advantages of both parental lines (short maturation time of TL7 and high resistance to soybean rust of ĐT2000) was selected and named Đ9 to introduce to the national official trials for release as a new commercial variant.

Đ9 was evaluated in national trials conducted by the National Center for Assessment of Plant Varieties and Products, Department of Crop Production, MARD in the winter of 2015, spring of 2016, and winter of 2016. Đ9 and its control DT84 were evaluated at seven experimental sites in six provinces located in northern and central regions of Vietnam. Experimental design and data collection were in accordance with the National Technical Regulation on Testing of Value of Cultivation and Use of Soybean varieties (QCVN 01-58:2011/BNN). Briefly, the experiments were conducted in a completely randomized block design with three replicates. Plot size was 10 m^2 (5 m × 2 m), the width of each plant bed surface was 1.4 m with four rows, and the distance between rows was 0.35 m. During 2017-2019, trials of the Đ9 cultivar were scaled up in different provinces. The experimental area for this step was 1.000 m^2/ cultivar/ site. Đ9 was officially approved by MARD for commercialization in northern Vietnam (Decision No. 337/QĐ-TT-CLT, October 16, 2019).

PERFORMANCE

Agronomic characteristics

Cultivar Đ9 displays a determinate growth phenotype, with a plant height of 42.6 cm in the winter crop season, and 66.4 cm in the spring season. The difference between seasons was larger than that of either parent. Đ9 produced from 3.0 to 3.7, branches on its main stem (Table 1). These plant structures enable Đ9 harvesting using a machine.

To expand soybean cultivation in Vietnam, desirable maturation times are approximately 85-90 days in the winter season, and approximately 100 days in the spring season. Days to maturity for Đ9 averaged 89 days (winter) and 100 days (spring). These times to maturity were between those of the two parents. Đ9 thus overcame the long maturation period (110 – 130 days) that limits cultivation of the male parent ĐT2000. The other general phenotypic characteristics of Đ9 were quite similar to those of its two parental varieties (Table 1).

Đ9 showed good performance in terms of yield, producing from 38.6 to 42.0 pods per plant with an average of 2.3-2.5 grains/pod. In combination with an average weight of 100 grains in the large category (17.5 g – 19.4 g), Đ9 expressed a potential for high grain yield.

In both winter 2015 and spring 2016, Đ9 and its parental lines were affected slightly by some common soybean diseases. Less than 10% of plants were observed to have dry root rot disease (caused by *Rhizoctonia bataticola* fungi); the score of soybean powdery mildew disease ranged from 1 to 3 (on a 5-point scale) and soybean downy mildew disease (*Peronospora manshurica*) scores ranged from 1 to 3 (on a 9 point scale) (Table 1).

Rust disease response

Đ9 was confirmed to possess the *Rpp2* resistance gene using the SSR marker *Satt620*. In the spring of 2016, Đ9 and its parental varieties, TL7 and ĐT2000, were subjected to an inoculation experiment, using two soybean rust isolates (IS-15 and IS-17) originating in northern and central Vietnam. The experiment was conducted at the Plant Protection Institute, MARD. The performance of Đ9 was comparable with that of ĐT2000- the parental line carrying the *Rpp2* and *Rpp4* resistance genes, with a low AUDPC score, and typical resistant phenotype with reddish-brown (RB) lesions. By contrast, the parental line TL7 was highly susceptible to both fungal isolates (Table 2, Figure 1).

Results of the official National VCU trial

Đ9 and DT84 (reference variety) were included in official VCU trials from 2015 to 2019. In the first trial, experiments were conducted on an experimental scale (each cultivar was planted in a plot of 10 m² with three replications) spanning three growing seasons: winter 2015, spring 2016, and winter 2016 in six provinces in northern and central Vietnam. Đ9 was similar to the reference variety in plant height and days to maturity. By contrast, Đ9 showed a higher potential for grain yield, with a higher number of fruits per plant, whereas the average grain weight was comparable to that of DT84. Both cultivars were naturally infected by some common fungal diseases at minor levels (Supplemental Table 10). The grain yield of Đ9 was significantly higher than that of DT84 in four of six experimental sites during the winter season, and in two of four sites during the spring season. The average difference ranged from 130 to 230 kg ha^{-1} in winter and was approximately 440 kg ha^{-1} in spring (Table 3).

In the second VCU trial, Đ9 and DT84 were evaluated in large-scale field experiments for two seasons, winter 2018 and spring 2019, in three provinces. The grain yield of Đ9 was higher than that of DT84 by 18.3% in Thai Nguyen, 17.8-22.1% in Bac Ninh, and 11.4- 14.8% in Thanh Hoa (Table 4). These results are the most important criteria underlying the decision to release Đ9 as a new cultivar for soybean production in northern Vietnam.
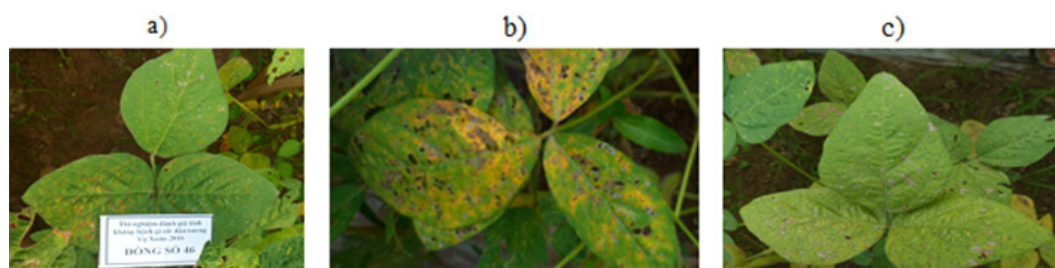

Figure 1. Reactions of Đ9 (a) and two parental lines TL7 (b) and ĐT2000 (c) in the inoculation experiment. TL7 showed a typical phenotype with TAN lesions, while lesions on Đ9 and ĐT2000 were RB.

Table 4. Results of VCU trial in large-scale field in winter 2018 and spring 2019

Experimental sites	Cultivar	Winter 2018			Spring 2019		
		Maturity (days)	**Grain yield** (100 kg ha^{-1})	**Difference from control** (%)	**Maturity** (days)	**Grain yield** (100 kg ha^{-1})	**Difference from control** (%)
Thai Nguyen	Đ9	90	23.2	18.3	98	26.5	18.3
	DT84	89	19.6		97	22.4	
Bac Ninh	Đ9	88	24.5	22.1	97	27.8	17.8
	DT84	87	20.8		97	23.6	
Thanh Hoa	Đ9	85	23.3	14.8	94	25.4	11.4
	DT84	83	20.3		93	22.8	

Registration and protection

Đ9 was registered and protected as a commercial cultivar by MARD decision (No. 337/QĐ-TT-CLT, signed on October 16, 2019), and the Self-announcement of Director of Field Crops Research Institute on commercializing a new soybean cultivar, Đ9 (No. 379/VCLT-KH, signed on November 23, 2020). The Field Crops Research Institute holds Đ9 grain production and commercialization rights in Vietnam. Đ9 seeds are available for exchange as breeding germplasms with breeders in other countries for research purposes.

Supplemental Tables can be accessed by e-mail tvquangnn1@gmail.com.

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