



## Identification Physiological Races of *Phakopsora pachyrhizi* Syd. Causing Asian Soybean Rust in India

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### ABSTRACT

The physiological variability studies were carried out for 17 isolates collected from different growing environment of India. Three pathotypes were identified based on physiological variability on 13 international set of differentials namely Cluster I-Reddish Brown (RB) lesion producing pathotypes, Cluster II- TAN lesion producing pathotypes and Cluster III- Mixed or RB + TAN producing pathotypes. Based on the reaction of *Phakopsora pachyrhizi* isolates on two differentials PI 230971 and PI 200492, Race 2 produced RB type lesions on PI 230971 and TAN type on the other differentials and Race 3 produced TAN type lesion PI 200492 and RB type lesion on the other differentials were identified. Based on reaction on these two differentials, Race 2 and Race 3 are prevalent in Karnataka state while, only Race 2 was prevalent in Maharashtra and north eastern states.

**KEYWORDS :** Soybean, *Phakopsora pachyrhizi* Syd, Pathotypes TAN lesion and RB lesions, Host differentials.

### Introduction:

Asian soybean rust caused by *P. pachyrhizi* Syd. is the potential disease causing severe losses in yield and also quality of oil in India. Most of the research on management of soybean rust has focused on screening of genotypes and the use of fungicides. Research on physiological variability of *P. pachyrhizi* Syd over different agroecological regions of the country is lacking. The soybean growers of the subcontinent are seriously facing the infestation of rust disease in the last two decades with a yield loss ranging from 30-80 per cent in many states such as Karnataka, Maharashtra, Manipur, Nagaland and Meghalaya. The information on prevalence of physiological races will pay way for developing stable genotypes with multiple resistance for more than one race in a particular geographical area. Physiological races of *P. pachyrhizi* also have been reported in Taiwan, Australia, China, and Japan. In Taiwan in 1983, 50 single-urediniospore isolates of *P. pachyrhizi* inoculated onto the five soybean entries—plant introduction (PI) 462312 (Ankur), TK-5, TN 4, PI 200492 (Komata), and PI 230971—were differentiated into three physiological races based upon specific reaction patterns of RB and TAN lesions (Yeh, 1983). In Australia, one race was virulent on soybean cv. Williams and avirulent on PI 200492, while another race was virulent on both soybean entries (McLean and Byth, 1977). In another study, six races of *P. pachyrhizi* were identified using 257 entries from six Glycine spp. (Burdon and Speer, 1984). In China, seven *P. pachyrhizi* isolates were differentiated into four races using a set of eight soybean entries (Tan and sun, 1989). In Japan, 18 *P. pachyrhizi* races were differentiated using 11 soybean entries based upon reaction type and the number of uredinia produced per lesion (Yamaoka et al., 2002).

Since there was no information on physiological races of Asian soybean rust under Indian conditions, the different infected samples were collected representing isolates of *Phakopsora pachyrhizi* of India. The results on prevalence of races are discussed in this paper which are important in developing durable and stable sources of resistance in contemporary soybean breeding programme.

### Materials and Methods

#### Collection of isolates and preservation

Different infected samples were collected representing isolates of *Phakopsora pachyrhizi* from different geographical areas of the country. For this study, twelve different locations of northern part of Karnataka (Bagalkot, Belgaum, Bidar, Dharwad and Haveri districts), two isolates from Maharashtra and three isolates from north eastern states

were collected from a susceptible variety JS 335. The rust spores were harvested immediately from the infected leaves. Spores were tapped and teased out from the pustules with the help of sterilized needle. Uredospores were collected in sterilized two ml centrifuge tubes, labeled and stored at -20°C in deep freezer for further studies.

The collected isolates were inoculated on different host differentials to study, variation in *P. pachyrhizi* isolates with respect to number of lesions, reaction type (nature of virulence) and lesion shape on the host both under laboratory and glass house conditions. Based on these observations pathotype clusters were formed for *P. pachyrhizi* isolates representing different geographical areas to know variation on host differentials.

#### Inoculation procedure

Prior to inoculation, stored uredospores were removed from deep freezer and heat shocked at 40°C for 5 min. Uredospores were hydrated overnight by floating them in a small centrifuge tubes on sterile distilled water. The inoculum of each isolate was prepared by suspending uredospores in 0.1% tween 20 (sodium monolaurate) in sterile distilled water, mixing vigorously and filtering through a spore filter cloth. The uredospores concentration was adjusted to 20,000 uredospores/ml for inoculation as described by Devaraj, 2012.

#### Reaction of different isolates under laboratory condition

A single leaf piece was carefully placed in a 9 cm diameter petri dish with adaxial side appressed on 1 per cent agar technical amended with kinetin at 10 µg/ml. Then, the abaxial leaf surfaces were sprayed with 400 µl of spore suspension of different isolates. The petri plates containing leaf were then incubated at 20°C with 12/12 hr light/dark cycles for 15 days. The observation on number of lesions, lesion shape and colour were recorded.

#### Reaction of different isolates under glasshouse condition

One day prior to inoculation, thirteen set of differentials at three to four leaf stage plants were covered with polythin cover to create congenial conditions for rust development. Uredospores of each isolate was inoculated to abaxial leaf surface by spray as well as staple method (Hegade et al., 2001). After inoculation again plants were covered with poly thin cover upto 10 days. After fifteen days of inoculation observations were recorded on number of lesion, lesion shape and colour of lesion.

**Results and discussion:**

The collected isolates were inoculated on different host differentials to study, variation in *P. pachyrhizi* isolates, number of lesions, reaction type and lesion shape on the host under both laboratory and glass house condition. The results are present in Table 1a to 4b. In laboratory condition, the number of lesions varied from 1 to 15 lesions / leaf in different isolates. The maximum number (15 lesions / leaf) was recorded on differential TK-5 for Varur isolate. The minimum number of lesion (1) was observed in most of the differentials for different isolates. Reaction type recorded based on the colour of the pustule produced on the leaf viz. reddish brown (RB) colour, TAN colour and mixed infection (RB+Tan). Maximum of 15 isolates exhibited TAN colour on TK-5, seven mixed infection (RB+Tan) on JS 335 and most of the reaction showed RB on differentials against different isolates. The observation taken on lesion shape recorded based on the pustule produced on the leaf viz. circular pustule (C), irregular pustule (I) and irregular to circular pustule were observed. Whereas maximum of fifteen irregular pustule were recorded on TK-5, six irregular pustules to circular pustule were recorded on EC 391160 and most of the reactions showed C on differentials against different isolates.

Based on the above observations the isolates were categorized into different clusters viz. I (RB lesion producing pathotypes), II (TAN lesion producing pathotypes) and III (RB+TAN producing pathotypes). Maximum of sixteen isolates on PI 230970 and PI 230971 differentials and minimum seven isolates on PI 459024B were grouped in cluster -I, similarly maximum of six isolates on PI 459024B and no isolate on PI 230970 in cluster -II. Seven isolates on JS 335 and one isolate on three differentials in cluster -III were recorded (Fig.4). Similar findings were reported from Yamaokal et al. (2002); Bonde et al. (2006); Pham et al. (2009); Twizeyimana et al. (2012); Devaraj (2012); Maphosa et al. (2013). Where, they reported a set of different pathotypes pertaining to different differentials.

In glasshouse condition, the number of lesions varied from 1 to 14 lesion / leaf in different isolates. The maximum number (14 lesions / leaf) was recorded on differential PI 459025B for Haveri isolate. The minimum number of lesion (1) was observed in most of the differentials for different isolates. Reaction type recorded based on the colour of the pustule produced on the leaf. Maximum of ten TAN colour lesions on TK-5 for Hirehonnalli, eight mixed infection (RB+Tan) on JS 335 and most of the reaction showed RB on differentials against different isolates. The observation taken on lesion shape recorded based on the pustule produced on the leaf viz. Circular pustule (CP), irregular pustule (IP) and irregular to circular pustule were observed. Whereas maximum of eight irregular pustules were recorded on JS 335, six IP and CP on TK-5 and most of the reactions showed CP on differentials against different isolates.

Based on the above observations the isolates were categorized into different clusters viz. I (RB lesion producing pathotypes), II (TAN lesion producing pathotypes) and III (RB+TAN producing pathotypes). Maximum of seventeen isolates on EC 241778 differentials and minimum six isolates on PI 459025F were grouped in cluster -I, similarly maximum six isolates on JS 335 and EC 462312, where no isolates were recorded on EC 241778 in cluster -II and four isolates on TK -5 and zero on three differentials in cluster -III (Fig.4). Similar findings were reported from, Yamaoka et al. (2002); Bonde et al. (2006); Bonde et al. (2008). Pham et al. (2009); Twizeyimana et al. (2012); Maphosa et al. (2013).

Based on the reaction on different host differentials under glasshouse condition, three infection types such as RB, TAN and mixed infection of RB + TAN were observed. For identification of the races, the only two differentials (PI 230971 and PI 200492) was considered as per reports of Yeh (1983). Based on the reaction of these two differentials two races of *Phakopsora pachyrhizi* were identified. Race 2 produced RB type lesions on PI 230971 and TAN type on the other differentials and Race 3 produced TAN type lesion on PI 200492 and RB type lesion on the other differentials. Based on reaction on these two differentials, Race 2 and Race 3 are prevalent in Karnataka state while, only Race 2 was prevalent in Maharashtra and north eastern states. Similar work carried out by Yeh (1983) based on the differential reaction, identified three races of *P. pachyrhizi*, Race 1 produced TAN type lesion on all the soybean cultivars, Race 2 produced RB type lesions on PI 230971 and TAN type on the other differentials and Race 3 produced TAN type lesion PI 200492 and RB type lesion on the other differentials. The dis-

tribution of the races varied among the 17 locations. Race 2 and Race 3 are common in Karnataka while, only Race 2 was common in Maharashtra and north eastern states. This is the first report of race pattern of *P. pachyrhizi* in India

Under field condition, all the thirteen differentials were examined to study the reaction at MARS Dharwad. The maximum severity grade (9) was recorded on PI 459024B, PI 200492, JS 335 and Wyne. These differentials recorded disease severity of 92.56 PDI. This also clearly showed variability of Dharwad isolate against differentials. The differentials PI 459024B, PI 200492, PI 459025F, EC 391160, PI 230970, PI 459025B, JS 335, TK -5 and Wyne showed highly sporulating type pustules in Dharwad conditions fig. 5 (Devaraj, 2012).

Based on the above observations the isolates were categorized into different clusters viz. I (RB lesion producing pathotypes), II (TAN lesion producing pathotypes) and III (RB+TAN producing pathotypes). Similar findings were reported from Pham et al. (2009); Twizeyimana and Hartman (2012); Devaraj (2012); Maphosa et al. (2013). Based on these observations pathotype cluster were created for *P. pachyrhizi* isolates representing different geographical areas to know variation on host differentials. Similar findings, were reported by Melching et al. (1979); Bonde and Brown (1980) and Burdon and Speer (1984). where, they reported a set of different pathotypes pertaining to different differentials. Thus the study reported prevalence of physiological race pattern of *P.pachyrhizi* in India.

**Table: 1a Reaction of Phakopsora pachyrhizi isolates on host differentials based on number of lesion per leaf in laboratory**

State	Isolates	Differentials												
		Lesions/leaf*												
		PI 459024B	PI 459025F	EC 241778	EC 241780	EC 391160	PI 230970	PI 200492	JS 335	PI 230971	PI 459025B	EC 462312	TK-5	Wyne
Karnataka	Akkolar (Aa)	2	4	1	4	6	5	24**	13	6	1	5	3	4
	Bidar (B)	6	14	2	3	23	2	1	3	1	5	3+1	2+4	
	Dharwad (Dh)	5	10	6+1	3	7	8	3	2	2	3+5	9	2	2+3
	Garap (Ga)	4	1+1	2	6	1	4	5+2	3+4	4	2	5	4	4
	Haveri (Ha)	2	10	2+2	5	5	7	2	5	1	9	3	7	6
	Hirehonnalli (Hh)	3+2	5	4	8	5	3	10	5+1	7	5	8	1	9
	Hukkeri (Hu)	1	3	8	5+1	6	2	2	5	3	10	6+4	3	2
	Kabbur (Kb)	2+2	2	3	1	3	1	1+3	3	1	2	1	1	1
	Mudhol (Mu)	3	1	3	2	3	2	1	5	2	4	3	2+2	5
	Nidholi (Ni)	5	6	2	2	2	2	3+2	6+2	2	6	2	1	2
Maharashtra	Usgahurd (Ug)	3	1	3	1	3+3	3+1	3	10+3	2	3	4	4	1
	Varur (Va)	1	6	2	7	6	9	2	2+1	1	4+2	2	15	4
	Maharashtra (Ma)	3	4	2	3+2	2	5	6	3+1	6	2	4	2	1
Manipur	Tung (Tu)	3	2	4	5	2+2	2	4	4	3	2	3	2	2
	Imphal (Im)	2+1	5	1+1	2	2	6	10	3+1	3	4	4+2	4	1
Meghalaya	Usham (Um)	9	11	2	2	4	1	12	1	4	1+1	2	3	3+2
	Meditopama (Me)	1+1	4	3	4	3	10	2	2	4	4	5	2	1

\*Average of five leaves \*\* Reddish brown + Tan lesion (mixed infection)

**Table 1b: Reaction of Phakopsora pachyrhizi isolates on host differentials based on number of lesion in glasshouse**

State	Isolates	Differentials												
		Lesions/leaf*												
		PI 459024B	PI 459025F	EC 241778	EC 241780	EC 391160	PI 230970	PI 200492	JS 335	PI 230971	PI 459025B	EC 462312	TK-5	Wyne
Karnataka	Akkolar (Aa)	4	1	1	4	8	2+2**	2	2	2	5	3	4	1
	Bidar (B)	2+2	2	2	2	3	1	2	3	2	4	9	5	2
	Dharwad (Dh)	3	4	2	4	2+2	6	1	3	2	4+1	7	2+1	4+5
	Garap (Ga)	2	2	3	1	3	1	4	4+3	2	3	4	4	3
	Haveri (Ha)	4	3+2	1	4	5	4	8	4+4	3	14	7	2	3
	Hirehonnalli (Hh)	4	3	2	2	3	4	10	3	2	2+2	8	3+2	4
	Hukkeri (Hu)	1+3	2	2	2	2	5	7	4	2	6	11	2	6
	Kabbur (Kb)	3	2	4	1	6	3	3	6	4	3	5	6	1+1
	Mudhol (Mu)	1+1	5	2	4	1	2	4	3	5	9	2+1	2	2
	Nidholi (Ni)	3	4	4	1	3	3+2	1	1	10	1	2	7	3
Maharashtra	Usgahurd (Ug)	2	4+2	4	3	5	1	1	2+2	7	2	2	3	6
	Varur (Va)	2	3	5	2	1+3	2	1	3	2+1	1	1	2	1
	Maharashtra (Ma)	2	2	1	3	4	3	3	6	2	3	2	3+3	3
Manipur	Tung (Tu)	2	4	5	3	2	3+3	6	8	2	7	6	2	1
	Imphal (Im)	2	6	2+1	2	5	3	3	1	3	4	3+1	3	2
Meghalaya	Usham (Um)	4	1+3	2	2	6	4	3	3	1	3	7	2+1	3
	Meditopama (Me)	1	3	3	1	5	2	3	5	4	2	2	5	4

\*15 days after inoculation \*\* Reddish brown + TAN lesion (mixed infection)

Table 2a: Reaction of Phakopsora pachyrhizi isolates on host differentials based on reaction type in laboratory

Table with columns: State, Isolates, Differentials (Reaction type leaf), and Wane. Rows include Karnataka (Akkalur, Bidar, Dharwad, Ganag, Hassan, Hirehonnalli, Hukkeri, Mudhol, Nidhoshi, Ugarkhur, Varur), Maharashtra (Marathwada, Tung), Manipur (Imphal), Meghalaya (Umiyam), and Nagaland (Medziphema).

\*15 days after inoculation Note: TAN- Tan colour RB- Reddish Brown Colour

Table 2b: Reaction of Phakopsora pachyrhizi isolates on host differentials based on reaction type in glasshouse

Table with columns: State, Isolates, Differentials (Reaction type leaf), and Wane. Rows include Karnataka (Akkalur, Bidar, Dharwad, Ganag, Hassan, Hirehonnalli, Hukkeri, Mudhol, Nidhoshi, Ugarkhur, Varur), Maharashtra (Marathwada, Tung), Manipur (Imphal), Meghalaya (Umiyam), and Nagaland (Medziphema).

Note: TAN- Tan colour, RB- Reddish Brown Colour

Table 3a: Reaction of Phakopsora pachyrhizi isolates on host differentials based on lesion shape in laboratory.

Table with columns: State, Isolates, Differentials (Lesion shape), and Wane. Rows include Karnataka (Akkalur, Bidar, Dharwad, Ganag, Hassan, Hirehonnalli, Hukkeri, Mudhol, Nidhoshi, Ugarkhur, Varur), Maharashtra (Marathwada, Tung), Manipur (Imphal), Meghalaya (Umiyam), and Nagaland (Medziphema).

Note: C- Circular lesion, I- Irregular lesion

Table 3b: Reaction of Phakopsora pachyrhizi isolates on host differentials based on lesion shape in glasshouse

Table with columns: State, Isolates, Differentials (Lesion shape), and Wane. Rows include Karnataka (Akkalur, Bidar, Dharwad, Ganag, Hassan, Hirehonnalli, Hukkeri, Mudhol, Nidhoshi, Ugarkhur, Varur), Maharashtra (Marathwada, Tung), Manipur (Imphal), Meghalaya (Umiyam), and Nagaland (Medziphema).

Note: C- Circular lesion, I- Irregular lesion

Table 4a: Grouping of Phakopsora pachyrhizi different isolates in various clusters (laboratory condition)

Table with columns: Differentials, Cluster-I, Cluster-II, Cluster-III. Rows list various isolates grouped into three clusters based on laboratory conditions.

Note:

Table with columns: Dharwad - Dh, Hirehonnalli - Hi, Varur - Va, Ugarkhur - Ug, Hukkeri - Hu, Kabbur - kb, Mudhol - Mu, Bidar - Bi, Haveri - Ha, Akkialur - Aa, Imphal - Im, Umiam - Um, Medziphema - Me, Marathwada - Ma, Garag - Ga, Nidhoshi - Ni.

Table 4b: Grouping of Phakopsora pachyrhizi different isolates in various clusters (glasshouse)

Table with columns: Differentials, Cluster-I, Cluster-II, Cluster-III. Rows list various isolates grouped into three clusters based on glasshouse conditions.

Note:

Table with columns: Dharwad - Dh, Hirehonnalli - Hi, Varur - Va, Ugarkhur - Ug, Hukkeri - Hu, Kabbur - kb, Mudhol - Mu, Bidar - Bi, Haveri - Ha, Akkialur - Aa, Imphal - Im, Umiam - Um, Medziphema - Me, Marathwada - Ma, Garag - Ga, Nidhoshi - Ni.

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