

# Morphological comparison of isolates of *Phakopsora pachyrhizi* from different locations of the Karnataka.

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**ABSTRACT:** In this study, morphological characteristics of uredospores of *Phakopsora pachyrhizi* Syd. the fungus causing rust disease in soybean has been studied with a view to determining the presence of morphological forms of the pathogen. Morphological characters such as shape, colour and size of uredospore (length and width) of *P. pachyrhizi* were studied. Characteristics of twenty three isolates of *P. pachyrhizi* showed considerable variability with respect to spore colour, spore shape and size (length and width). Six different coloured uredospores were observed viz., dark Brown, light pale yellow, pale yellow, pale brown to colourless, light to dark brown and orange brown.

Ellipsoidal, irregular and circular shape was observed in twenty three isolates of *P. pachyrhizi* with different size. The length ranged from 92.93 to 63.41 µm and the width ranged from 73.05 to 45.01 µm. Ugarkhurid isolate was found to be different among 23 different isolates of *P. pachyrhizi* with respect to size, shape and colour.

**Key Words:** Soybean, *Phakopsora pachyrhizi*, isolates and morphology.

## INTRODUCTION

Spore characteristics have been extensively used in fungi to distinguish different morphological forms of a pathogen. Yang (1977) studied the morphology of soybean rust and reported that uredospores are short hyaline to yellowish-brown sub globose, ovate or oblong, 15 to 24 x 15 to 34 µm in diameter, with a finely echinulated hyaline wall 1 to 1.5 µm thick. Bonde and Brown (1980) examined isolates of soybean rust from Australia, India, Philippines, Taiwan and Puerto Rico on cultivar Wayne. Isolates were indistinguishable in their pre and post penetration, colonization phases and morphology of uredinial stage. The use of urediospores in determining morphological forms in rust fringe is quite common, apparently because of the relative ease with which they can be obtained. In contrast, it is not always possible to get plentiful teliospores and hence these are less frequently employed in the recognition of morphological forms. Studies on variability of pathogens have a greater significance in breeding for resistance as well as documenting resistant source/s. Most of the varieties and germplasm which are being grown on commercial scale are found to be susceptible to soybean rust. One of the reasons for lack of substantial durable resistance in the material may be attributed to presence of variability in the population (Patil, 2004). In order to develop the disease resistant and high yielding cultivars, it is imperative to analyse and understand the variability of the pathogen (Kapooria, 1973).

## Materials and methods

Soybean leaf samples showing typical symptoms of rust were collected from northern part of Karnataka state (Belgaum, Dharwad, Haveri, and Bagalkot districts). Two hundred fifty gram of leaf sample was collected from each location/cultivar. Leaves showing severe infection with maximum rust pustules were collected and stored at -20 °C in deep freezer for further studies. The rust samples of soybean were labeled based on the location of collection with two alphabets as mention in Table 1. The uredospores were scrapped from the infected leaf tissue of soybean and chickpea with help of a sterile scalpel and teased in a drop of lactophenol on glass slide and viewed under microscope in 40 x 10 X magnification of. The microphotographs were taken with the help of the Olympus digital camera attached to the microscope (U-25 ND 25, T2, Japan). This work was carried out in laboratory of AICRP on Soybean, UAS Dharwad.

The unit of measurement was fixed to micrometers (µm) and random 10 photographs of the spore mass were taken. The software has an inbuilt tool to measure the length of microscopic objects. Spore colours, shape, size (lengths and widths) of 100 spores were measured. The data was put in the Microsoft excel sheet, which was programmed to calculate the dimensions, to get an average value. The average/mean value was regarded as the representative uredospore dimension of a particular isolate. Morphological characters such as shape of uredospore, colour of uredospore and size of uredospore (length and width) of population of isolates of *Phakopsora pachyrhizi* were studied. Colour of uredospore was compared with standard colour of red, green and blue custom value of M.S. Office of computer.

Table 1.1:

Colour	Shape	Size (µm)		Circle (µm)		Irregular (µm)		Efficacy	
		Length	Width	Circum.	Radius	Width	Circum.		
DB	I and E	69.83	32.96	-	-	65.66	217.25	67.66	35.08
DB	C and E	65.54	51.08	178.29	28.37	-	-	-	36.46
DB	I and E	64.72	55.31	190.66	30.34	60.5	215.52	62.5	-
DB	I and E	77.04	34.71	-	-	66	244.46	84.5	38.17
LPV	C and E	68.95	55.85	208.85	33.25	-	-	-	38.82
PV	I and E	84.61	53.44	-	-	64.5	225.39	64.2	-
PBC	I and E	74.53	50.95	-	-	64.75	207.76	61.75	41.03
PBC	C and E	73.47	69.01	243.36	38.73	-	-	-	42.27
LPV	I and E	77.05	45.01	-	-	57.00	213.69	64.00	39.05
DBB	I and E	65.92	51.66	-	-	56.00	193.68	66	-
LDB	I and E	66.52	39.66	-	-	68.99	204.42	61.3	-
PV	I and E	74.99	54.17	-	-	-	-	-	39.83
LDB	C and E	82.70	66.91	235.03	37.40	-	-	-	44.62
OB	C and E	74.42	38.27	212.93	33.88	-	-	-	38.45
LDB	C and E	67.59	56.15	197.73	31.47	-	-	-	34.78
DB	I and E	77.23	50.34	-	-	61.00	233.56	78.00	41.75
PV	C and E	85.60	64.10	222.34	35.38	-	-	-	43.30
PBC	C and E	69.38	54.94	205.91	32.77	-	-	-	36.01
LDB	C and E	73.16	55.08	215.21	34.25	50.00	201.58	73	41.60
DB	C and E	69.95	52.84	209.64	33.26	-	-	-	38.02
LDB	C and E	92.93	73.05	-	-	-	-	-	49.27
PV	C and E	63.41	45.01	210.15	33.44	-	-	-	-
DB	I and E	75.56	51.68	-	-	91.00	233.95	50.99	37.50
		74.04	55.81	210.88	33.54	64.12	216.47	66.73	39.83
Age (Max.)		92.93	73.05	243.36	38.73	91.00	244.46	84.5	49.27
(Min.)		63.41	45.01	178.29	30.34	50.00	193.68	50.99	35.08

DB- Dark Brown, LPV-Light Pale Yellow, PV- Pale Yellow, PBC- Pale brown to colourless, LDB- Light to dark brown, OB- Orange Brown

**Table 1:** Designation of isolates of *Phakopsora pachyrhizi* Syd. obtained from various locations

## Result and Discussion

Morphological characters such as shape, colour and size of uredospore (length and width) of *P. pachyrhizi* were studied. Characteristics of twenty three isolates (designated abbreviation of villages) of *P. pachyrhizi* are described in Table 2. These isolates showed considerable variability with respect to spore colour, spore shape and size (length and width). Ono et al. (1992) also studied two species for the soybean rust fungus. It was based on morphological differences between their anamorphic and teleomorphic

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Received: July 3<sup>rd</sup> 2020, Accepted: July 10<sup>th</sup> 2020, Published: July 29, 2020



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stages which was based primarily on laying of telia and uredinia.

**Colour of spore**

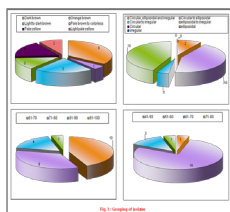
Colour of the uredospore compared with standard colour of red, green and blue colour custom value of computer. The value of different colour is given in Appendix I. Six different coloured uredospores were observed viz., dark Brown, light pale yellow, pale yellow, pale brown to colourless, light to dark brown, orange brown. Dark brown colour was common among the isolates (Table 2a). Dark brown colour was observed in eight isolates and only one isolate showed orange brown which was from Mudhol region. Sarboy et al. (1972) studied the uredospore morphology of soybean rust and recorded that, uredia were hypophyllous, scattered, sub epidermal eruptent causing minute lesions on the upper side, light cinnamon to dirty brown in colour, pulverulent, 100 to 200 µ in diameter.

Colour of isolates	Dark brown	Orange brown	Light to dark brown	Pale brown to colourless	Pale yellow	Light pale yellow
No. of isolates	8	1	5	3	4	2

Table2: Grouping of isolates on the basis of spore shape

**Shape of spore**

Three shapes viz., ellipsoidal, irregular and circular were observed in twenty three isolates of *P. pachyrhizi*. Most of them were with circular to ellipsoidal (10) or ellipsoidal to irregular shape (10), very few isolates with shape of circular to ellipsoidal to irregular (2) and circular to irregular (1) Table 2 b and Fig.1. Among the isolates, ellipsoidal shape frequency was maximum (52%) followed by irregular (29%) and circular (19 %) Table 2 d. Yang (1977) studied the morphology of soybean rust and reported that uredospores are short hyaline to yellowish-brown sub globose, ovate or oblong,



Shape of isolates	Circular, ellipsoidal and irregular	Circular to ellipsoidal	Circular to irregular	ellipsoidal to irregular	Circular	ellipsoidal	irregular
No. of isolates	2	10	1	10	-	-	-

Table3: Grouping of isolates on the basis spore size

**Size of uredospore**

All isolates of different geographical area differed in size, the length ranged from 92.93 to 63.41 µm and the width ranged from 73.05 to 45.01 µm (Table 2). The mean value of length was 74.04 µm and mean width was 55.81 µm. On the basis of length and width range twenty three isolates grouped in four categories. For length maximum isolates (10) were in the range between 60-70 µm (Table 2c) followed by 70-80 µm (8). The highest length was found in Ugarkhurd isolate (92-93 µm) and lowest length was observed in Vaderkatti isolate (63.41) which belongs to Bailhongal area (Table 2, Fig.1). Similarly Carmona (2005) identified The two spore types that were commonly observed were urediniospores and teliospores of *P. pachyrhizi*. Telia were found on infected leaves mixed with uredinia in every sample. Urediniospores measured 16 to 22 µm (mean 18.5 µm) × 25 to 30 µm (mean 27 µm). Teliospores measured 8 to 11 µm (mean 9 µm) × 19 to 27 µm (mean 23.8 µm).

Size of isolates	Length (µm)				Width (µm)			
	61-70	71-80	81-90	91-100	41-50	51-60	61-70	71-80
No. of isolates	10	8	4	1	1	18	3	1

Sixteen isolates came under the width range between 50-60 µm followed by 60-70 µm (3) (Table 2c Fig.1). The highest width was found in Ugarkhurd isolate (73.05 µm) and Kallur isolate showed lowest width (45.01 µm) (Table 2). Based on circular, free form and ellipsoidal shape of isolates their circumference, radius, width and length were calculated and presented in Table 2. Morphological variability of 23 isolates revealed that Ugarkhurd isolate was found to be different from other isolates which has recorded highest length (92-93 µm) and width (73.05µm) of uredospore. Variability in colour was also seen in different geographical isolates with Ugarkhurd isolate. Three different types of shape were observed within the isolates and there was variability in shape among the isolates.

Sr. No.	Isolates	Shape of spore	Frequency *		
			Circular	Ellipsoidal	Irregular
1.	Aa.	Irregular and ellipsoidal	-	57	43
2.	At.	Circular and ellipsoidal	23	77	-
3.	Ba.	Circular and irregular	30	-	70
4.	Bi.	Irregular and ellipsoidal	-	72	28
5.	Dh.	Circular and ellipsoidal	31	69	-
7.	Ga.	Irregular and ellipsoidal	-	36	64
6.	Ha.	Ellipsoidal and irregular	17	-	83
8.	Hi.	Circular and ellipsoidal	37	63	-
9.	Ka.	Irregular and ellipsoidal	-	73	27
10.	Kn.	Irregular and ellipsoidal	-	30	70
11.	Ko.	Irregular and ellipsoidal	-	43	57
12.	Ma.	Irregular and ellipsoidal	-	22	78
13.	Mr.	Circular and ellipsoidal	35	65	-
14.	Mu.	Circular and ellipsoidal	59	41	-
15.	Na.	Circular and ellipsoidal	34	66	-
16.	Ni.	Irregular and ellipsoidal	-	43	57
17.	Ra.	Circular and ellipsoidal	42	58	-
18.	Ry.	Circular and ellipsoidal	23	77	-
19.	Sa.	Circular, ellipsoidal and irregular	17	43	40
20.	Si.	Circular and ellipsoidal	21	79	-
21.	Ug.	Circular, ellipsoidal and irregular	12	60	28
22.	Va.	Circular and ellipsoidal	36	64	-
23.	Ya.	Irregular and ellipsoidal	-	67	33
<b>Total</b>			<b>417</b>	<b>1195</b>	<b>678</b>
<b>Percent frequency</b>			<b>19</b>	<b>52</b>	<b>29</b>

\*One hundred spores observed.

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