



Pathogenic behaviour of leguminous isolates of *Rhizoctonia solani* collected from different Indian agro-ecological regions

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ABSTRACT

Pathogenic behaviour of 368 isolates of *Rhizoctonia solani* representing seven anastomosis groups collected from different leguminous crops from 16 agro-ecological regions of India was tested on mungbean (cv. Ratna), urdbean (cv. Barabanki local), cowpea (cv. V 578) and chickpea (cv. JG 62) under artificially inoculated conditions in pots. The isolates were highly variable in virulence and caused 10% to 100% wet root rot incidence in mungbean, urdbean and cowpea and 11% to 100% in chickpea. The isolates obtained from agro-ecological region 15 (western Himalayas, warm sub-humid ecoregion) caused significantly higher disease incidence in mungbean, whereas the isolates from agro-ecological region 10 (central highlands, hot sub-humid ecoregion) caused significantly higher disease incidence in urdbean and cowpea. In chickpea, the isolates from agro-ecological region 16 (Assam and Bengal plains, hot humid ecoregion) caused maximum disease incidence. The virulence of the isolates was also analyzed according to their crops of origin. Isolates from lentil caused the highest disease incidence in mungbean. Single isolate of rice bean caused the highest disease incidence in urdbean and cowpea, whereas kidney bean isolates caused the highest disease incidence in chickpea. The highest numbers of isolates covering maximum number of agro-ecological regions were obtained from mungbean followed by urdbean, cowpea and chickpea. One hundred thirteen isolates of the pathogen isolated from diseased roots were selected for aerial inoculation to test their ability to cause the aerial infection. All the isolates proved to be pathogenic to mungbean by aerial inoculation and caused 10%–50% disease incidence. The study clearly indicates that the pathogen is able to cause infection to the roots as well as aerial parts of the leguminous plant. The virulence of the isolates did not correspond to their region of origin, host, parts of plant and anastomosis groups.

Key words: Agro-ecological region, Cross pathogenicity, Leguminous crops, *Rhizoctonia solani*, Wet root rot

India grows a variety of pulse crops under a wide range of agro-ecological conditions and is recognized globally as a major player in pulse production contributing 25.4% of the global production. The compound growth rate of yield of pulses (0.67) is lower as compared to that of the cereals (2.15). This has resulted in the decline in per capita availability of pulses from 71 g in 1955 to 41.8 g in 2008 necessitating their import to meet the domestic demands. To alleviate protein and energy malnutrition, a minimum of 50 g pulses/capita/day should be available in addition to other sources of protein. To make up this shortfall in supply and to meet the additional demand from the burgeoning population, at least 23.9 mt of pulses are required by 2015. This figure is expected to touch 29.3 mt by 2020 (Agricultural Statistics at a Glance 2009). The per cent contribution of pulses in total foodgrain production in India has declined during the last three decades

owing to low-endowed land and biotic constraints. Around 8–10% production is lost annually because of the ravages of diseases alone costing nearly ₹ 10 000 million (Vishwadhar and Chaudhary 2001).

Rhizoctonia solani Kühn [teleomorph - *Thanatephorus cucumeris* (Fr.) Donk] is a destructive soil-borne plant pathogen (Saksena and Dwivedi 1973) infecting a wide range of agricultural and horticultural crops, including legumes, worldwide causing several diseases (Gonzalez *et al.* 2006). The pathogen causes considerable yield loss in mungbean and urdbean in India (Dubey 2003). Yield loss up to 57% in mungbean was reported from Iran (Kaiser 1970). It is likely that the population of *R. solani* in soil has gradually built-up new disease problems under changed conditions of intensive cultivation and agro-practices. As a collective species, *R. solani* is genetically diverse showing variability in respect of pathogenicity as well as cultural/morphological and physiological characters. Initially, *Rhizoctonia* spp are classified in different species or groups on the basis of morphological and cultural characters (Parmeter and Whitney 1970). A method based on anastomosis groups (AGs) has

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been used for identification and classification of *R. solani* (Parmeter *et al.* 1969, Ogoshi 1987). Although AGs and pathogenicity are related, to some extent, evidence from several studies indicated considerable pathogenic variation between strains from within AG (Ogoshi 1987). Understanding of the disease epidemiology and host-pathogen interaction is greatly dependent on the knowledge of the diversity of pathogen at field level. Very limited studies on the virulence characterization and grouping of the host-specific isolates of *R. solani* have been carried out so far. Attempt has not been made yet to classify and characterize the isolates of *R. solani* obtained from various leguminous crops on the basis of pathogenic behaviour/virulence and to correlate this with agro-ecological regions, hosts of origin and AGs. Keeping these points in view, the present study was aimed to determine the virulence of the populations of *R. solani* representing various AGs associated with various leguminous crops in different agro-ecological regions of India having diverse cropping sequences.

MATERIALS AND METHODS

The samples were collected during the winter and rainy seasons of 2007 and 2008 from 16 agro-ecological regions of India covering 18 states for collecting diseased specimens of major leguminous crops and weeds present in leguminous fields. One to seven states from each agro-ecological region, one to two districts within a state and two to five villages in a district were surveyed based on crops under cultivation as well as appearance of the disease. Two fields were selected randomly for the collection of diseased specimens and the disease incidence in these fields was also recorded. Total 21 states and 62 districts were surveyed for collection of diseased specimens.

Plant samples showing characteristic symptoms of the disease as web blight/wet root rot were collected and processed for isolation. The leaves and stems of mungbean, urdbean, other beans and weeds showing infection of the pathogen were processed for isolation. For chickpea, pea and lentil, infected roots were processed because the pathogen causes only wet root rot symptoms. Standard procedure was followed for isolation of pathogen on 2% potato dextrose agar (PDA) medium. The pathogenicity of the isolated fungus was tested on the respective hosts and re-isolation was made to confirm the pathogenic nature of the isolates. The isolated fungus was identified as *Rhizoctonia solani* Kühn on the basis of their morphological characters as described by Parmeter and Whiteny (1970) with *Thanatephorus cucumeris* (Fr.) Donk as its perfect state (Talbot 1970). Three hundred sixty eight pathogenic isolates of *R. solani* were obtained from different agro-ecological regions (Table 1) and cultures of the isolates were kept in refrigerator (4°C) for further use.

All 368 isolates representing 7 different AGs (AG 1, AG2-2, AG 2-2LP, AG 2-3, AG 3, AG 4 and AG 5) were studied for their pathogenic behaviour on major leguminous hosts, namely, cowpea [*Vigna unguiculata* (L.) Walp.],

mungbean [*Vigna radiata* (L) Wilczek], urdbean [*Vigna mungo* (L.) Hepper] and chickpea (*Cicer arietinum* L.). The experiments were conducted in net house under artificially inoculated condition during rainy and winter seasons of 2008–09 and 2009–2010 for respective crops. Surface sterilized (0.1% formalin) plastic pots (15 cm diameter) were filled (2 kg/pot) with sterilized soil (1.0% formalin). The soil was inoculated 2 days prior sowing with 10-day-old inoculum (20 g/pot) of *R. solani* multiplied on sorghum grains. Sorghum grains were soaked in tap water for 12 hr, strained and filled into 500 ml conical flasks. The flasks containing grains were autoclaved for two subsequent days at 1.1 kg/cm² for 30 min. and inoculated with 2-day-old culture of the *R. solani*. The inoculated flasks were incubated at 25 ± 1°C for 10 days. Ten seeds of urdbean (cv. Barabanki local), mungbean (cv. Ratna), cowpea (cv. V 578) and chickpea (JG 62) were sown in each pot in three replications separately for each isolate of *R. solani*. Wet root rot incidence was recorded 45 days after sowing in mungbean, urdbean and cowpea while 60 days after sowing in chickpea.

One hundred thirteen isolates of *R. solani* isolated from diseased roots of various leguminous crops were selected for aerial inoculation to test their ability to cause aerial infection. Surface sterilized plastic pots were filled with sterilized soil as mentioned earlier and each pot was sown with 10 seeds of mungbean (cv. Ratna). Twenty-day-old plants were inoculated with 10-day-old inoculum of *R. solani* multiplied on typha (*Typha latifolia* L) stem pieces. Stem pieces (2–3 cm) colonized with *R. solani* were placed on aerial part of the plant and maintained relative humidity by irrigating the pot and 2–3 times spraying of sterilized water on the foliage of the inoculated plants. Seven days after inoculation, the disease incidence was recorded.

RESULTS AND DISCUSSION

Three hundred sixtyeight isolates of *R. solani* were isolated from different leguminous hosts collected from 16 agro-ecological regions of India. The incidence of the disease varied from 2 % to 47 % and they were differentiated into low, medium and high incidence areas. The agroecological regions 4, 9, 11, 15 and 20 showed high disease incidence (>15%), whereas regions 2, 3, 5, 12, 13, 14 and 16 showed medium (10-15%) incidence. The regions 6, 7, 8 and 10 showed low (up to 10%) disease incidence. Maximum numbers of isolates were obtained from agro-ecological region 4 (northern plain and central highlands, hot semi-arid ecoregion) from maximum number of crops followed by regions 2 and 14. Only three isolates were obtained from agroecological region 10 (Table 1).

The pathogenic behaviour of 368 isolates representing 16 agro-ecological regions of India and 11 leguminous hosts was tested on mungbean, urdbean, cowpea and chickpea. The isolates were highly variable in respect of their virulence and in general no host specificity was observed. On mungbean, urdbean and cowpea, the disease incidence caused

Table 1 Place of collection of different isolates of *R. solani* their crop of origin and anastomosis groups

Place of collection		No. of isolates	Crops	Anastomosis groups
Agroecological region (AR)	State			
AR2-Western plains, hot arid ecoregion	Haryana, Punjab and Rajasthan	41	Mungbean, urdbean, cowpea, chickpea, rice bean and weeds	AG 1, AG 2-3, AG 4 and AG 5
AR3-Deccan plateau, hot arid ecoregion	Karnataka	6	Mungbean	AG 3
AR4-Northern plains and central highlands, hot semi-arid ecoregion	Rajasthan, Delhi, Madhya Pradesh, Haryana, Uttar Pradesh, Punjab, Gujarat	59	Mungbean, urdbean, cowpea, chickpea, lentil and weeds	AG 1, AG 2-2 LP, AG 2-3, AG 3, AG 4 and AG 5
AR5-Central (Malwa) highlands and Kathiawar peninsula, hot semi-arid ecoregion	Gujarat	25	Mungbean, urdbean, cowpea, chickpea, and weeds	AG 2-2, AG 2-3, AG 3 and AG 5
AR6-Deccan plateau, hot semi-arid ecoregion	Karnataka, Andhra Pradesh and Maharashtra	35	Mungbean, pea and chickpea	AG 1, AG 2-2, AG 2-3 and AG 5
AR7-Deccan plateau and eastern Ghat, hot semi-arid ecoregion	Andhra Pradesh	13	Chickpea and soybean	AG 1 and AG 5
AR8-Eastern ghat (TN uplands) and Deccan plateau, hot semi-arid ecoregion	Tamil Nadu	6	Urdbean and chickpea	AG 1
AR9-Northern plains, hot sub-humid ecoregion	Uttarakhand, Uttar Pradesh and Punjab	31	Mungbean, urdbean, cowpea, pea, lentil and weeds	AG 1, AG 2-2 and AG 3
AR10-Central highlands, (Malwa and Bundelkhand), hot sub-humid ecoregion	Madhya Pradesh	3	Chickpea and pea	AG 1, AG 2-3, AG 4
AR11-Deccan plateau and central highlands (Bundelkhand), hot sub-humid ecoregion	Madhya Pradesh and Maharashtra	31	Mungbean, urdbean, chickpea and weeds	AG 2-3, AG 3 and AG 4
AR12-Eastern plateau (Chhattisgarh region), hot sub-humid ecoregion	Uttar Pradesh	12	Mungbean, urdbean, chickpea and pea	AG 2-2 and AG 5
AR13-Eastern (Chhota Nagpur) plateau and eastern ghats, hot sub-humid ecoregion	Jharkhand and Odisha	8	Mungbean, urdbean and cowpea	AG 1, AG 2-2, AG 2-3, AG 3, AG 4 and AG 5
AR14- Eastern plains, hot sub-humid ecoregion	Uttar Pradesh	40	Urdbean, cowpea and kidneybean	AG 2-3 and AG 3
AR15- Western Himalayas, warm sub-humid ecoregion	Jammu and Kashmir, Uttarakhand and Himachal Pradesh	22	Mungbean, urdbean, soybean and French bean	AG 1, AG 2-3, AG 2-2 LP and AG 5
AR16- Asom and Bengal plains, hot humid ecoregion	Asom	30	Cowpea	AG 2-2, AG 2-3, and AG 4
AR20- Western ghat and coastal plains, hot humid per humid ecoregion	Kerala	6	Cowpea	AG 2-3

by all isolates varied from 10–100% while on chickpea it was ranged from 11–100%. The wet root rot incidence caused by all the isolates was analyzed according to agro-ecological region. (Table 2) The isolates obtained from agro-ecological region 15 (western Himalayas, warm sub-humid ecoregion) caused the highest disease incidence on mungbean, followed

by the isolates obtained from agro-ecological regions 4, 5, 9, 13 and 14 with statistically similar disease incidence. The isolates obtained from agro-ecological region 10 central highlands (Malwa and Bundelkhand), hot sub-humid ecoregion] caused the highest disease incidence on urdbean and cowpea, followed by region 9 (northern plains, hot sub-

Table 2 Disease incidence caused by the legume isolates of *Rhizoctonia solani* collected from different agro-ecological region on mungbean, urdbean, cowpea and chickpea

Agroecological region	Isolate (no.)	Crops (no.)	Disease incidence (%) on different crops			
			Mungbean	Urdbean	Cowpea	Chickpea
AR 2	41	6	64.2 (53.3) ^c	76.6 (61.1) ^e	68.3 (55.7) ^k	70.8 (57.3) ^e
AR 3	6	1	33.3 (35.3) ^g	53.3 (46.9) ^l	70.0 (56.8) ^j	76.6 (61.1) ^c
AR 4	59	6	71.4 (57.7) ^b	67.8 (55.4) ⁱ	79.7 (63.2) ^e	73.0 (58.7) ^d
AR 5	25	5	72.4 (58.3) ^b	68.4 (55.8) ^{ij}	64.8 (53.6) ^m	61.6 (51.6) ^j
AR 6	35	3	58.6 (49.9) ^{cde}	74.9 (59.9) ^f	81.1 (64.3) ^d	56.0 (48.5) ^l
AR 7	13	2	31.5 (34.2) ^g	68.5 (55.8) ⁱ	53.8 (47.2) ⁿ	65.0 (53.7) ^h
AR 8	6	2	48.3 (44.1) ^f	68.3 (55.8) ^{ij}	73.3 (58.9) ^h	54.0 (47.3) ^m
AR 9	31	6	71.9 (58.0) ^b	64.9 (68.5) ^b	76.8 (61.2) ^f	67.1 (55.0) ^f
AR 10	3	2	63.3 (52.7) ^c	90.0 (71.6) ^a	100 (90.0) ^a	66.0 (54.3) ^g
AR 11	31	4	64.2 (53.3) ^c	78.9 (66.6) ^e	74.2 (59.5) ^g	64.2 (53.3) ⁱ
AR 12	12	4	62.5 (52.2) ^{cd}	60.0 (50.8) ^k	66.7 (54.7) ^l	47.0 (43.3) ⁿ
AR 13	8	3	71.3 (57.6) ^b	70.0 (56.8) ^h	70.0 (56.8) ^j	61.1 (51.5) ^j
AR 14	40	3	73.5 (59.1) ^b	69.0 (56.2) ⁱ	71.8 (57.9) ⁱ	66.2 (54.5) ^g
AR 15	22	4	78.6 (62.5) ^a	71.8 (57.9) ^g	89.1 (70.7) ^c	78.2 (62.2) ^b
AR 16	30	1	51.3 (45.8) ^{de}	81.7 (64.7) ^d	90.3 (72.0) ^b	90.1 (71.6) ^a
AR 20	6	1	50.0 (45.0) ^f	71.7 (57.9) ^g	76.7 (61.1) ^f	59.3 (50.4) ^k

The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test. Figures in parentheses are transformed angular values.

AR2- Western plains, hot arid ecoregion, AR3- Deccan plateau, hot arid ecoregion, AR4- Northern plains and central highlands, hot semi-arid ecoregion, AR5- Central (Malwa) highlands and Kathiawar peninsula, hot semi-arid ecoregion, AR 6- Deccan plateau, hot semi-arid ecoregion, AR7- Deccan plateau and eastern Ghat, hot semi-arid ecoregion, AR8- Eastern ghat (TN uplands) and Deccan plateau, hot semi-arid ecoregion, AR9- Northern plains, hot sub-humid ecoregion, AR10- Central highlands, (Malwa and Bundelkhand), hot sub-humid ecoregion, AR11- Deccan plateau and central highlands (Bundelkhand), hot sub-humid ecoregion, AR12- Eastern plateau (Chhattisgarh region), hot sub-humid ecoregion, AR13- Eastern (Chhota Nagpur) plateau and eastern ghats, hot sub-humid ecoregion, AR14- Eastern Plain, hot sub-humid ecoregion, AR15- Western Himalayas, warm sub-humid ecoregion, AR16- Asom and Bengal plains, hot humid ecoregion, and AR20- Western ghat and coastal plains, hot humid per humid ecoregion. Samples were not collected from regions number AR1, AR17, AR18, AR19 and AR21.

humid ecoregion) for urdbean and region 16 (Asom and Bengal plains, hot humid ecoregion) for cowpea. The isolates obtained from the region 16 caused the highest incidence in chickpea, followed by the region 15. Therefore, the isolates from agro-ecological regions 10 and 16 caused the maximum disease incidence on the crops evaluated in the present study. Out of 368 isolates, 62.8% isolates proved to be highly pathogenic on mungbean, 79.4% on urdbean, 83.4% on cowpea and 75.6% on chickpea with >50% disease incidence. The isolates caused >20 to 50% disease incidence were considered as moderate pathogenic and 29.9% isolates for mungbean, 16% isolates for urdbean, 10.3% isolates for cowpea and 20.6% isolates for chickpea were placed in this category. Minimum numbers of isolates were proved to be weak pathogenic on mungbean (7.3%), urdbean (4.6%), cowpea (6.3%) and chickpea (3.8%). Cowpea was found to be more susceptible, followed by urdbean and mungbean.

The isolates were also belonging to seven different anastomosis groups (AGs) as AG 1, AG 2-2, AG 2-2LP, AG 2-3, AG 3, AG 4 and AG 5. The frequencies of AG 3, followed by AG 2-3 were more among of the isolates

evaluated in the present study. The AG groups were not corresponding to the virulence on the host. Erper *et al.* (2011) reported that AG 4 was the most common group of *R. solani* on bean and soybean crops. The pathogenicity tests on bean and soybean seedlings showed that the highest disease severities were caused by AG 4 isolates, whereas AG 1 and AG 6 isolates were moderately pathogenic. In present study, all AGs showed more or less same pathogenic behaviour on the crops evaluated. The incidence of the disease was more in northern parts of India; hence more number of isolates was originated from this area. Maximum numbers of isolates were obtained from agro-ecological region 4, but the isolates originated from region 10 and 15 caused more disease incidence in comparison to that of region 4 on the crops tested. The majority of the isolates were highly pathogenic to mungbean, urdbean, cowpea and chickpea. Of these crops, cowpea was more susceptible as compared to urdbean, mungbean and chickpea. The virulence of the isolates was not corresponding to their crop origin. The present findings clearly indicated the potential of the isolates of *R. solani* isolated from leguminous hosts to cause serious disease in

leguminous crops. Further, this study demonstrated the relative potential of the various *R. solani* AGs and particularly AG 1, AG 2-2, AG 2-2 LP, AG 2-3, AG 3, AG 4 and AG 5 also prove a significant threat to leguminous crops grown in various agro-ecological regions of India. The present findings were accordance with the observations made by You *et al.* (2008) while testing of cross-pathogenicity of *R. solani* strains on pasture legumes. This is a first report on the occurrence of these AGs on leguminous hosts in India. The variation in respect of virulence among the isolates of same AG was also observed. Ogoshi (1987) also observed pathogenic variation between strains of an AG.

Analysis of disease incidence caused by the isolates on different crops indicated that maximum numbers of isolates were obtained from mungbean, followed by urdbean, cowpea and chickpea. Lentil isolates of the pathogen caused the highest disease incidence on mungbean followed by kidney bean isolates. Rice bean isolates caused the highest disease incidence on urdbean and cowpea followed by lentil and pea isolates. Kidney bean isolates produced the highest disease incidence on chickpea followed by lentil isolates. Irrespective of the origin of the crops, the isolates caused the highest disease incidence in cowpea (Table 3). The isolates of *R. solani* isolated from diseased roots of various leguminous crop plants were proved pathogenic on aerial parts of mungbean plants and caused 10–50% disease incidence.

The isolates obtained from roots of the leguminous crops also proved pathogenic on aerial parts of the mungbean indicating the non-host part specificity of the pathogen, although the isolates obtained from aerial plant parts caused more severe aerial infection in comparison to that of the isolates obtained from the roots. Thus, it was evident that the pathogen could cause the disease on roots as well as on the aerial part of the plants. The present findings are supported

by the observations of Mikhail *et al.* (2010) that the grouping of the isolates of the *R. solani* obtained from cotton based on their virulence pattern was not related to their geographic origin, AG or host. *R. solani* AG 2-2 has been reported to cause rot on legumes (Sweetingham *et al.* 1986) and soybean (Fenille *et al.* 2004). Besides, legumes it was reported on other host crops also. AG 3 is a common on potato tubers (Cubeta and Vilgalys 2000) and the most genetically traceable member of the *R. solani* species complex and is a pathogen that affects important food crops in the plant family Solanaceae (Ceresini *et al.* 2007) was also amongst the population evaluated in the present study. The AG 3 and AG 5 have been reported from India on potato (Thind and Aggarwal 2008). Interestingly, besides AG 3, AG 5 was also isolated from leguminous host and was found to be pathogenic on mungbean, urdbean, cowpea and chickpea during virulence test. Prevalence of different AGs on leguminous hosts may be due to the difference in the cropping sequences and weather variables present in various agro-ecological regions of India. The pathogen has evolved in such a way under these conditions to cause the diverse diseases in leguminous crops along with other crops of the area.

The present study clearly indicates that the populations of *R. solani* found to be associated with leguminous hosts in India were highly variable in their virulence and the virulence of the isolates was not corresponding to the agro-ecological regions of their origin, hosts, parts of plant and AGs.

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Table 3 Disease incidence caused by the isolates of *Rhizoctonia solani* collected from different leguminous crops on mungbean, urdbean, cowpea and chickpea

Crop	Isolate (no.)	Agro-ecological region (no.)	Disease incidence (%) on different crops			
			Mungbean	Urdbean	Cowpea	Chickpea
Mungbean	117	10	66.7 (54.7) ^e	67.1 (55.0) ⁱ	74.7 (59.8) ^h	67.4 (55.2) ^c
Urdbean	72	10	74.7 (59.8) ^d	70.3 (57.0) ^h	77.1 (61.4) ^f	66.1 (54.4) ^f
Cowpea	71	8	61.5 (51.7) ^h	72.1 (58.1) ^e	75.6 (60.4) ^e	76.2 (60.8) ^c
Chickpea	60	9	49.0 (44.5) ⁱ	78.3 (62.3) ^c	77.7 (61.8) ^e	66.4 (54.6) ^f
Soybean	11	2	45.5 (42.4) ^j	65.5 (54.0) ^j	58.2 (49.8) ^j	57.4 (49.2) ^e
Pea	10	4	76.0 (60.7) ^c	80.0 (63.4) ^b	84.0 (66.4) ^c	47.0 (43.3) ^j
Kidneybean	10	1	81.0 (64.2) ^b	74.0 (59.4) ^f	78.0 (62.0) ^e	80.5 (63.8) ^a
Lentil	2	2	85.0 (67.2) ^a	80.0 (63.4) ^b	90.0 (71.6) ^b	79.0 (62.7) ^b
Ricebean	1	1	70.0 (56.8) ^f	90.0 (71.6) ^a	100.0 (90.0) ^a	50.0 (45.0) ^h
Frenchbean	3	1	73.3 (59.0) ^e	76.7 (61.1) ^d	80.0 (63.4) ^d	69.5 (56.5) ^d
Weeds	11	5	74.5 (59.7) ^d	75.3 (60.1) ^c	69.1 (56.2) ^j	66.4 (54.6) ^f

The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test. Figures in parentheses are transformed angular values

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