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Differential response of F_4 and F_5 green gram [*Vigna radiata* (L.) Wilczek] recombinant inbred lines (RILs) to powdery mildew infection

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Abstract
Green gram is severely affected by powdery mildew caused by fungus *Erysiphe polygoni* D.C. The present investigation involved F_4 and F_5 generations of RILs of two crosses *viz*., Chinamung × BL-849 and Chinamung × LM-1668 which has contrasting response for powdery mildew resistance with 146 and 155 lines respectively. Out of 146 F_4 RILs screened in cross Chinamung × BL-849, one of them was found to be highly resistant (R0) *viz.*, C1-34-23. When screened in Rabi season (F_5) showed fourteen RILs to be R0. Whereas in cross Chinamung × LM-1668 155 F_4 RILs screened, thirty nine RILs were found to be moderately resistant (R2) whereas in Rabi season (F_5) showed one hundred and four R2. Similar resistance response by the lines like C1-34-23 in cross Chinamung × BL-849 and C2-14-11, C2-16-13 RILs in cross Chinamung × LM-1668 in both seasons clearly indicates that these lines have become stable for the disease response. Such lines can be screened for yield related traits to develop highly resistant breeding lines with high yielding ability in green gram.

Keywords: Green gram, Recombinant Inbred Lines, Powdery Mildew, Percent Disease Index

Introduction
Green gram [*Vigna radiata* (L.) Wilczek] with a diploid chromosome number of 2n=2x=22, belongs to the family Fabaceae, is an economically important *Vigna* crop species of Asia, widely grown in South and South-east Asia of the World (Kang *et al*., 2014) [2]. It is native to the Indo-Burma region with India, Burma, Thailand and Indonesia contributes almost 90% of the world's production (Raturi *et al*., 2014). For green gram cultivation, well drained loam to sandy loam soil is good, while saline and alkaline soil or waterlogged soils are not good for cultivation. Green gram mature seeds are low in anti-nutritional factors and highly digestible. It causes less flatulence as compared to other pulses, making it suitable for consumption, especially for children and old people. Green gram starch is considered to have a low glycaemic index and hence, the most suitable source of food for diabetic patients (Swaminathan *et al*., 2012) [5]. In spite of the best efforts for improving the green gram varieties, the yield potential of this crop remains low owing to biotic and abiotic factors. Powdery mildew caused by the fungus *Erysiphe polygoni* D.C., an obligate pathogen with a wide host range, is the most devastating foliar disease of green gram. It is favoured by dry season and cool weather conditions which in turn results in yield reduction ranging from 9-50%. Severe infection can reduce the yield between 20-40% and a complete 100% if infected at the seedling stage (Bainade *et al*., 2014) [1]. The pathogen survives in conidial form on various hosts in the off season. The disease is secondarily spread through air by conidia produced in the season. Breeding for disease resistance is the best way to avoid severe losses. Considering all the premises, an investigation was planned to evaluate F_4 and F_5 recombinant inbred lines of green gram for resistance to powdery mildew under field conditions.

Materials and Methods
In this study, F_4 and F_5 recombinant inbred line (RIL) population of two crosses *viz*., Chinamung × BL-849 and Chinamung × LM-1668, contrasting to powdery mildew resistance with 146 and 155 lines respectively were screened for resistance to powdery mildew. The parents differ for powdery mildew response as shown in Table 1.

Table 1: The characteristic features of parents and check varieties (*) of green gram.

Parents	Disease reaction	Disease Score
Chinamung (Female)	Susceptible	4
BL 849 (Male)	Highly Resistant	0
LM 1668 (Male)	Highly Resistant	0
*Pusa Baisakhi	Moderately Susceptible	3
*KKM-3	Moderately Susceptible	3

The experiment was conducted during *Kharif* 2015 and *Rabi* 2015 in Augmented Design with two checks *i.e.* Pusa Baisakhi, KKM-3 and parents *i.e.* Chinamung, BL-849 and LM-1668. Each genotype was grown with a spacing of 30 cm × 10 cm. All the RILs of both the crosses were evaluated for powdery mildew infection in both the seasons. Seeds from randomly selected single plant of every recombinant inbred lines were taken and forwarded to next generation of RILs. Powdery mildew disease intensity was recorded from the day of infection till harvest at weekly interval and the disease was scored on 0-5 scale as recommended by Reddy *et al*., (1994) [4] (Table 2). The score recorded based on the scale was converted into *per cent* disease index (PDI) and used for screening. Disease severity was calculated as PDI to know the extent of damage caused by the disease by following formula:

Per cent disease index

$$= \frac{\text{Sum of all disease rating} \times 100}{\text{Total number of ratings} \times \text{Maximum score}}$$

Table 2: Powdery mildew scale (Reddy *et al.,* 1994) [4]

Score	*Per cent* of leaf area infested	Disease reaction
0	0	Highly resistant (R0)
1	0.1-5	Resistant (R1)
2	5.1-30	Moderately Resistant (R2)
3	30.1-65	Moderately susceptible(MS)
4	65.1-90	Susceptible (S)
5	90.1-100	Highly susceptible (HS)

The one-way analysis of variance (ANOVA) was also used to verify the efficiency of scale, used for powdery mildew disease scoring into different classes based on *per cent* disease index (PDI) values.

Results

In this investigation, an attempt was made to identify recombinant inbred lines resistant to powdery mildew disease in green gram. The results revealed considerable amount of variability among RILs for powdery mildew resistance. Out of 146 F_4 RILs screened for powdery mildew under field condition in cross Chinamung × BL 849, one of them was found to be highly resistant (R0) *viz.*, C1-34-23. Seventy five RILs were found to be moderately resistant (R2). Sixty four were found to be moderately susceptible (MS), four were susceptible (S) and two were highly susceptible (HS) *viz.*, C1-451-247 and C1-501-292. While similar 146 F_5 RILs screened in Rabi season showed fourteen RILs to be R0. One hundred and nine were R2 and twenty four were found to be MS (Table 5). Whereas in cross Chinamung × LM 1668, out of 155 RILs screened for powdery mildew in field condition, thirty nine RILs were found to be R2. Eighty five were found to be MS and thirty one found to be S. While similar 155 F_5 RILs screened in Rabi season showed one hundred and four to be R2. Forty seven were MS and four were found to be S type (Table 6). The varying responses of each RIL screened for powdery mildew over two seasons are presented in Fig. 1 and Fig. 2. One-way analysis of variance revealed that the scale used for classification is highly significant thus conferring the reliability of scale suggested by Reddy *et al*., 1994 [4] (Table 3 and Table 4).

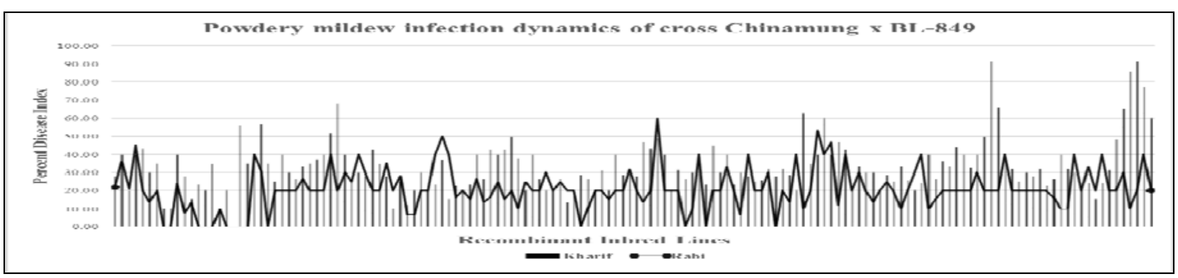

Fig 1: Powdery mildew infection dynamics of cross Chinamung × BL-849 in green gram.

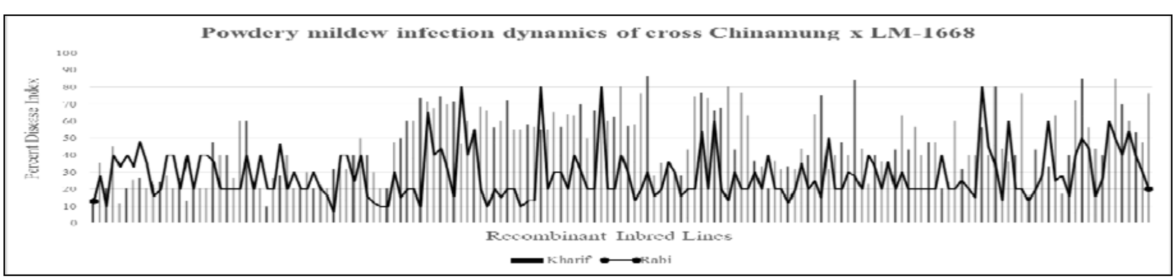

Fig 2: Powdery mildew infection dynamics of cross Chinamung × LM-1668 in green gram.

Table 3: Single factor analysis of variance for powdery mildew to check the significance of scale in F_4 and F_5 RILs of the cross Chinamung × BL 849 of green gram.

Source of variation	Chinamung × BL 849							
	F_4				F_5			
	Df	Mean sum of squares	F	P-value	Df	Mean sum of squares	F	P-value
Between Groups	4	6306.27**	132.53	9.63E-47	2	7881.09**	271.66	1.97E-49
Within Groups	141	47.58**			143	29.01**		
Total	145				145			

Table 4: Single factor analysis of variance for powdery mildew to check the significance of scale in F_4 and F_5 RILs of the cross Chinamung × LM 1668 of green gram.

Source of variation	Chinamung × LM 1668							
	F_4				F_5			
	Df	Mean sum of squares	F	P-value	Df	Mean sum of squares	F	P-value
Between Groups	2	23631.36**	327.09	8.5E-56	2	13958.27**	344.98	3.15E-57
Within Groups	152	72.25**			152	40.46**		
Total	154							

Discussion

The results speculate the different RILs of both the crosses present variable response to powdery mildew in two seasons due to quantitative nature of disease. As shown in Fig. 3 and Fig. 4, RILs have variable response per disease score in two seasons. Thus, the population can be used for QTL mapping for dissecting the nature of gene action controlling the disease. However, there is possibility of the RIL populations to be unstable so both the populations should be advanced for further generation so that they become stable. Some of the lines in two populations are showing similar response in both the seasons (Table 5 and Table 6) which clearly indicates that these lines have become stable for the disease response. Such lines which are showing stable resistant response to the powdery mildew can be screened for yield related traits and should be recommended for the development of highly resistant breeding lines for powdery mildew disease with high yielding ability in green gram.

Acknowledgement

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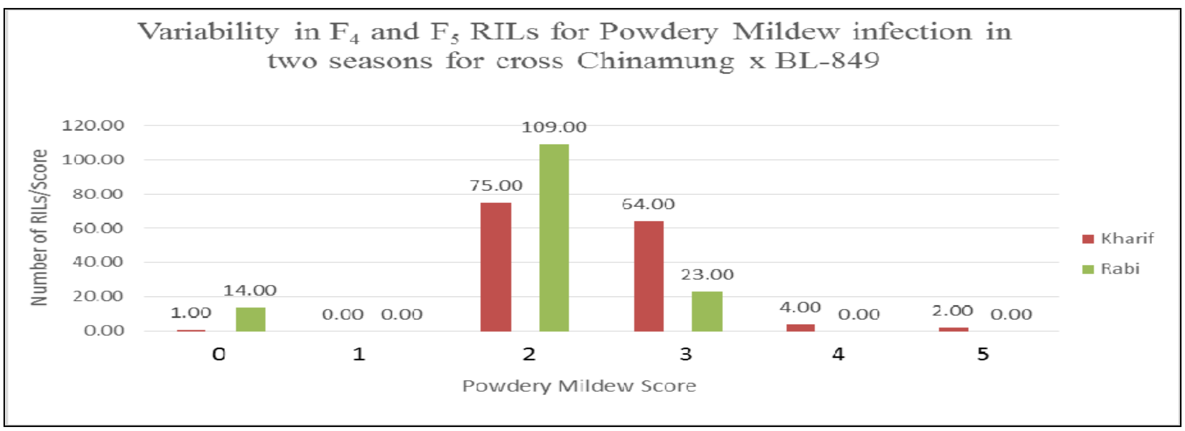

Fig 3: Varying response of F_4 and F_5 RILs for powdery mildew infection in two seasons for cross Chinamung × BL-849.

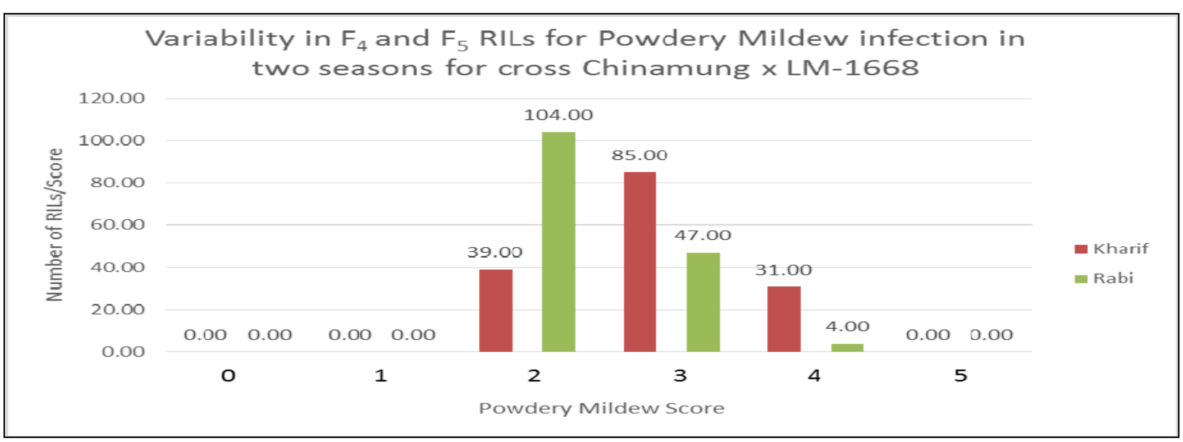

Fig 4: Varying response of F_4 and F_5 RILs for powdery mildew infection in two seasons for cross Chinamung × LM-1668

Table 5: Field screening of 146 F_4 and F_5 RILs for powdery mildew resistance of the cross Chinamung × BL 849 in green gram.

Grade	Leaf Area Infected (%)	Disease Reaction	RILs		Total RILs (146)		RILs showing same disease response in F_4 and F_5
			F_4	F_5	F_4	F_5	
0	0	Highly Resistant (R0)	C1-34-23	C1-15-10, C1-15A-11, C1-21A-17, C1-25-19, C1-28-20, C1-32-22, C1-37-23, C1-38-27, C1-41-28, C1-44-31, C1-175-111, C1-236-152, C1-246-159, C1-275-177	1	14	C1-34-23
1	1.1-5	Resistant (R1)	0	0	0	0	
2	5.1-30	Resistant (R2)	C1-9-6, C1-15-10, C1-15A-11, C1-16A-13, C1-17A-14, C1-21A-17, C1-25-19, C1-30-21, C1-32-22, C1-46-32, C1-50A-35, C1-51A-36, C1-78-56, C1-83-58, C1-84-59, C1-89-62, C1-91-64, C1-98-65, C1-102-66, C1-124A-67, C1-104-68, C1-106-69, C1-112-73, C1-122-79, C1-123-80, C1-132-87, C1-135-89, C1-128-85, C1-158-99, C1-161-101, C1-165-102, C1-168-105, C1-169-106, C1-172-109, C1-173-110, C1-175-111, C1-182-116, C1-185-119, C1-195-126, C1-205-132, C1-208-134, C1-214-137, C1-229-148, C1-236-152, C1-241-155, C1-246-159, C1-251-163, C1-260-168, C1-266-170, C1-267-171, C1-268-172, C1-270-174, C1-275-177, C1-283-182, C1-284-183, C1-404-205, C1-408-209, C1-410-211, C1-412-213, C1-413-214, C1-414-215, C1-416-217, C1-417-218, C1-422-221, C1-428-227, C1-463-258, C1-467-262, C1-470-265, C1-472-267, C1-474-268, C1-479-273, C1-481-275, C1-482-276, C1-487-281, C1-488-282	C1-1-1, C1-9-6, C1-13-7, C1-16-12, C1-16A-13, C1-17A-14, C1-30-21, C1-43-30, C1-46-32, C1-48-34, C1-50A-35, C1-51A-36, C1-52-37, C1-65-49, C1-63-47, CI-72-51, C1-75-54, C1-77-55, C1-78-56, C1-84-59, C1-86-60, C1-88-61, C1-91-64, C1-98-65, C1-102-66, C1-124A-67, C1-104-68, C1-109-69, C1-123-80, C1-132-87, C1-135-89, C1-131-86, C1-128-85, C1-145-93, C1-147-94, C1-148-95, C1-149-96, C1-154-98, C1-158-99, C1-160-100, C1-161-101, C1-165-102, C1-168-105, C1-169-106, C1-172-109, C1-173-110, C1-182-116, C1-185-119, C1-187-120, C1-195-126, C1-196-127, C1-206-132, C1-212-137, C1-217-139, C1-218-140, C1-226-146, C1-229-148, C1-232-150, C1-241-155, C1-247-160, C1-251-163, C1-260-168, C1-266-170, C1-268-172, C1-270-174, C1-274-176, C1-279-179, C1-283-182, C1-289-187, C1-295-190, C1-301-196, C1-404-205, C1-406-207, C1-408-209, C1-410-211, C1-412-213, C1-413-214, C1-414-215, C1-415-216, C1-416-217, C1-417-218, C1-423-222, C1-428-227, C1-429-228, C1-430-229, C1-435-233, C1-445-241, C1-446-242, C1-448-244, C1-450-246, C1-451-247, C1-454-250-, C1-461-256, C1-463-258, C1-467-262, C1-470-265, C1-471-266, C1-472-267, C1-474-268, C1-477-271, C1-478-272, C1-481-275, C1-487-281, C1-489-283, C1-490-284, C1-497-289, C1-500-291, C1-501-292, C1-510-296	75	109	C1-9-6, C1-16A-13, C1-17A-14, C1-30-21, C1-46-32, C1-50A-35, C1-51A-36, C1-78-56, C1-84-59, C1-91-64, C1-98-65, C1-102-66, C1-124A-67, C1-104-68, C1-106-69, C1-123-80, C1-132-87, C1-135-89, C1-128-85, C1-158-99, C1-161-101, C1-165-102, C1-168-105, C1-169-106, C1-172-109, C1-173-110, C1-182-116, C1-185-119, C1-195-126, C1-205-132, C1-214-137, C1-229-148, C1-241-155, C1-251-163, C1-260-168, C1-266-170, C1-268-172, C1-270-174, C1-283-182, C1-404-205, C1-408-209, C1-410-211, C1-412-213, C1-413-214, C1-414-215, C1-416-217, C1-417-218, C1-428-227, C1-463-258, C1-467-262, C1-470-265, C1-472-267, C1-474-268, C1-481-275, C1-487-281
3	30.1-65	Moderately Susceptible (MS)	C1-1-1, C1-13-7, C1-16-12, C1-28-20, C1-38-27, C1-41-28, C1-42-29, C1-43-30, C1-44-31, C1-48-34, C1-52-37, C1-65-49, C1-63-47, CI-72-51, C1-36A-53, C1-77-55, C1-86-60, C1-88-61, C1-120-	C1-42-29, C1-36A-53, C1-83-58, C1-89-62, C1-112-73, C1-120-78, C1-122-79, C1-208-134, C1-220-142, C1-242-156, C1-256-165, C1-267-171, C1-284-183, C1-296-191, C1-298-193, C1-300-	64	23	C1-42-29, C1-36A-53, C1-120-78, C1-220-142, C1-242-156, C1-256-165, C1-457-252

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4	65.1-90	Susceptible (S)	C1-75-54, C1-454-250, C1-500-291, C1-506-295	0	4	0	
5	90.1-100	Highly Susceptible (HS)	C1-451-247, C1-501-292	0	2	0	

Table 6: Field screening of 155 F_4 and F_5 RILs for powdery mildew resistance of the cross Chinamung × LM-1668 in green gram.

Grade	Leaf Area Infected (%)	Disease Reaction	RILs		Total RILs (155)		RILs showing same disease response in F_4 and F_5
			F_4	F_5	F_4	F_5	
0	0	Highly Resistant (R0)	0	0	0	0	
1	1.1-5	Resistant (R1)	0	0	0	0	
2	5.1-30	Resistant (R2)	C2-8-6, C2-9-7, C2-10-8, C2-12-9, C2-13-10, C2-14-11, C2-16-13, C2-19-14, C2-22-16, C2-23-17, C2-33-24, C2-35-26, C2-45-30, C2-50-33, C2-59-41, C2-69-47, C2-75-49, C2-79-51, C2-1289A-53, C2-82-54, C2-87-57, C2-88-58, C2-89-59, C2-90-60, C2-91-61, C2-92-62, C2-119-86, C2-1584-93, C2-136-95, C2-98-68, C2-103-73, C2-104-74, C2-1417-188, C2-1453-212, C2-1483-235, C2-1489-238, C2-1540-255, C2-1547-257, C2-1560-261	C2-14-11, C2-16-13, C2-23-17, C2-35-26, C2-55-38, C2-58-40, C2-59-41, C2-63-43, C2-69-47, C2-79-51, C2-1289A-53, C2-86-56, C2-87-57, C2-88-58, C2-89-59, C2-90-60, C2-91-61, C2-92-62, C2-93-63, C2-112-79, C2-118-85, C2-119-86, C2-1584-93, C2-136-95, C2-150-101, C2-1587-102, C2-153-104, C2-1586-105, C2-1203-108, C2-1215-115, C2-1224-123, C2-1225-124, C2-1229-127, C2-1230-128, C2-1232-130, C2-1233-131, C2-1234-132, C2-1236-134, C2-1237-135, C2-1253-142, C2-1255-144, C2-1256-145, C2-1257-146, C2-1259-148, C2-1260-149, C2-1261-150, C2-1268-155, C2-1269-156, C2-1278-161, C2-1280-163, C2-1281-164, C2-1284-167, C2-98-68, C2-1582-69, C2-103-73, C2-104-74, C2-105-75, C2-1290-169, C2-1294-172, C2-1296-174, C2-1297-175, C2-1406-179, C2-1409-181, C2-1412-184, C2-1413-185, C2-1416-187, C2-1418-189, C2-1419-190, C2-1421-191, C2-1432-196, C2-1438-199, C2-1439-200, C2-1444-203, C2-1447-206, C2-1448-207, C2-1449-208, C2-1451-	39	104	C2-14-11, C2-16-13, C2-23-17, C2-35-26, C2-59-41, C2-69-47, C2-79-51, C2-1289A-53, C2-87-57, C2-88-58, C2-89-59, C2-90-60, C2-91-61, C2-92-62, C2-119-86, C2-1584-93, C2-136-95, C2-98-68, C2-103-73, C2-104-74, C2-1540-255, C2-1541-256, C2-1547-257, C2-1560-261, C2-1572-267, C2-1573-268

				210, C2-1452-211, C2-1455-214, C2-1457-216, C2-1458-217, C2-1460-219, C2-1462-220, C2-1469-225, C2-1480-233, C2-1482-234, C2-1489-238, C2-1490-239, C2-1492-241, C2-1493-242, C2-1494-243, C2-1531-249, C2-1533-251, C2-1537-253, C2-1540-255, C2-1541-256, C2-1547-257, C2-1557-259, C2-1560-261, C2-1561-262, C2-1572-267, C2-1573-268, C2-1580-274, C2-1581-275			
3	30.1-65	Moderately Susceptible (MS)	C2-54-37, C2-55-38, C2-58-40, C2-63-43, C2-65-44, C2-86-56, C2-93-63, C2-106-76, C2-108-77, C2-112-79, C2-1585A-82, C2-118-85, C2-150-101, C2-1587-102, C2-153-104, C2-1586-105, C2-1219-118, C2-1220-119, C2-1221-120, C2-1229-127, C2-1230-128, C2-1233-131, C2-1234-132, C2-1236-134, C2-1237-135, C2-1242-139, C2-1253-142, C2-1255-144, C2-1256-145, C2-1257-146, C2-1258-147, C2-1260-149, C2-1268-155, C2-1269-156, C2-1278-161, C2-1280-163, C2-1582-69, C2-102-72, C2-105-75, C2-1406-179, C2-1412-184, C2-1413-185, C2-1416-187, C2-1418-189, C2-1419-190, C2-1421-191, C2-1432-196, C2-1433-197, C2-1438-199, C2-1439-200, C2-1446-205, C2-1447-206, C2-1448-207, C2-1449-208, C2-1452-211, C2-1454-213, C2-1455-214, C2-1456-215, C2-1457-216, C2-1458-217, C2-1460-219, C2-1462-220, C2-1469-225, C2-1480-233, C2-1482-234, C2-1490-239, C2-1492-241, C2-1493-242, C2-1494-243, C2-1498-245, C2-1499-246, C2-1531-249, C2-1532-250, C2-1533-251, C2-1541-256, C2-1556-258, C2-1557-259, C2-1561-262, C2-1567-265, C2-1572-267, C2-1573-268, C2-1574-269, C2-1578-272, C2-1579-273, C2-1580-274	C2-8-6, C2-9-7, C2-10-8, C2-12-9, C2-13-10, C2-19-14, C2-22-16, C2-33-24, C2-45-30, C2-50-33, C2-54-37, C2-65-44, C2-75-49, C2-82-54, C2-106-76, C2-108-77, C2-1585A-82, C2-1207-110, C2-1208-111, C2-1210-112, C2-1212-113, C2-1220-119, C2-1221-120, C2-1258-147, C2-1270-157, C2-102-72, C2-1291-170, C2-1295-173, C2-1417-188, C2-1433-197, C2-1446-205, C2-1453-212, C2-1454-213, C2-1456-215, C2-1483-235, C2-1499-246, C2-1530-248, C2-1532-250, C2-1556-258, C2-1565-263, C2-1566-264, C2-1567-265, C2-1574-269, C2-1575-270, C2-1577-271, C2-1578-272, C2-1579-273	85	47	C2-54-37, C2-65-44, C2-106-76, C2-108-77, C2-1585A-82, C2-1220-119, C2-1221-120, C2-1258-147, C2-102-72, C2-1433-197, C2-1446-205, C2-1454-213, C2-1456-215, C2-1499-246, C2-1532-250, C2-1556-258, C2-1578-272, C2-1579-273
4	65.1-90	Susceptible (S)	C2-1203-108, C2-1207-110, C2-1208-111, C2-1210-112, C2-1212-113, C2-1215-115, C2-1224-123, C2-1225-124, C2-1232-130, C2-1259-148, C2-1261-150, C2-1263-152, C2-1270-157, C2-1281-164, C2-1284-167, C2-1290-169, C2-1291-170, C2-1294-172, C2-1295-173, C2-1296-174, C2-1297-175, C2-1409-181, C2-1444-203, C2-1451-210, C2-1530-248, C2-1537-253, C2-1565-263, C2-1566-264, C2-1575-270, C2-1577-271, C2-1581-275	C2-1219-118, C2-1242-139, C2-263-152, C2-1498-245	31	4	C2-1263-152
5	90.1-100	Highly Susceptible (HS)	0	0	0	0	

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