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STOMATAL DENSITY AND CHLOROPHYLL CONCENTRATION AS AN INDICATOR OF POWDERY MILDEW RESISTANCE IN PEA (*Pisum sativum* L.)

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Powdery mildew significantly hampers pea yield and deteriorate its quality as well. The resistance breakdown remained the main reason for failure in each attempt to incorporate powdery mildew resistance in well adapted pea cultivars. A precise selection of powdery mildew resistant source is the key to develop high yielding and stable powdery mildew resistant cultivars. Field screening alone is not an efficient method for making selection for powdery mildew resistance. Different physiological parameters *viz.,* the concentration of total chlorophyll, chlorophyll b, chlorophyll a: chlorophyll b and the number of stomata were found to be in a tight positive correlation with disease severity while, the concentration of chlorophyll a had negative correlation. The differential behaviour of these traits in powdery mildew susceptible and resistant genotypes has suggested their use as reliable criteria for screening against powdery mildew. A 1-5 scale has been devised for screening against powdery mildew in pea; where score 1 is for highly resistant and 5 for highly susceptible genotype. The suggested scale is reliable and reproducible in making selections for powdery mildew resistance.
Keywords: Biotrophs, disease screening, *Erysiphe pisi,* legumes, resistance breakdown

INTRODUCTION

Plant diseases always remained major threat to human food and clothing requirements. The outbreak of different plant diseases was the major cause of famines in the recent past (Agrios, 1988). Pea being rich in protein contents is considered as an important member of legume crops. Pea is a cheap alternative of animal proteins for malnourished peoples of developing countries (Cousin *et al*., 1985; Azmat *et al.,* 2011). Like other field crops pea is also the victim of a range of diseases; in majority of the pea growing areas fungal diseases are more common (Hagedorn, 1985; Azmat *et al.,* 2012; Azmat and Khan, 2014). Powdery mildew caused by *Erysiphe pisi* is the major limitation for the yield and quality of pea harvest (Gritton and Ebert, 1975; Dixon, 1978; Azmat *et al.,* 2012). The incidence of this disease significantly reduces the seed yield ranging from 25-50% and the situation becomes adverse under hot and humid climate which may lead to complete failure of crop (Munjal *et al*., 1963; Gritton and Ebert, 1975; Thompson and Kelly, 1982). The use of fungicide is the main strategy to cope with the fatalities of powdery mildew (Ahmed *et al*., 2006). The application of fungicides is neither environment friendly nor effectively controlling the disease owing to the reason that the pathogen has evolved resistance against fungicides (Azmat *et al.,* 2012). Therefore, there is need to develop powdery mildew resistant cultivars to have long-term and effective control over the menace. The resistance to powdery mildew is under genetic control regardless of the controversy regarding the nature and number of gene(s) controlling the resistance (Tiwari *et al.,* 1997; Fondevilla *et al.,* 2010; Azmat *et al.,* 2010). The development of powdery mildew resistant cultivar is usually a lengthy procedure taking 8-12 years (Poehlman and Sleper, 1995); the selection of resistant plants with confidence is therefore crucial. At present field screening alone is done for the selection of resistant plants which is though easy, but not a reliable method. Keeping in view the limitations associated with field screening experiments and the importance of the effective selection of resistant plants the authors have devised an effective screening strategy. In addition to field screening, an effort was made to work out the application of stomatal density and chlorophyll concentration for the precise selection of powdery mildew resistant plants in pea.

MATERIALS AND METHODS

***Plant material and inoculation with powdery mildew*:** A total of 30 pea genotypes belonging to the same maturity group were selected on the basis of their response to powdery mildew (Azmat *et al*., 2012). The experimental material was comprised of 19 highly resistant and 11 highly susceptible pea genotypes (Table 1). The seeds of all the genotypes were surface sterilized with 2% hypochlorite and

Table 1. The physiological response of pea genotypes to powdery mildew.

Genotype	Disease Score (% leaf area affected)	Number of stomata		Size of Stomata (µm)		Chlorophyll (µg g^{-1})				Carotenoid (µg g^{-1})	
						a		b			
		α^	β>	α	β	α	β	α	β	α	β
It-96	0 (No infection)	52*	39	30×20	30×20	1156	1140	465	459	483	488
		64+	57	34×25	34×25						
20171	1 (<1%)	54	41	31×20	31×20	1151	1145	463	460	467	459
		67	60	30×24	30×24						
19782	2 (4%)	55	44	30×21	30×21	1230	1217	496	487	520	517
		63	59	35×23	35×23						
No. 380	3 (7%)	58	46	31×21	31×21	1264	1260	510	505	545	553
		67	62	33×20	33×20						
18293	5 (21%)	73	60	32×20	32×20	1597	1375	649	772	707	710
		68	65	34×22	34×22						
No. 267	0 (No infection)	50	40	31×20	31×20	1163	1145	468	457	475	473
		63	55	34×24	34×24						
10609	1(<1%)	53	43	31×21	31×21	1232	1210	497	489	556	549
		67	59	33×23	33×23						
10612	2 (4%)	58	46	32×22	32×22	1252	1243	505	501	575	577
		69	60	34×23	34×23						
9057	3(8%)	58	49	32×20	32×20	1271	1267	513	506	520	527
		67	63	33×21	33×21						
18412	4 (13%)	61	55	32×21	*34×23*	1276	1143	515	649	524	513
		70	62	33×23	*35×24*						
19727	4 (17%)	59	57	31×22	*33×22*	1295	1150	523	639	606	598
		67	64	33×24	*36×24*						
19616	6 (55%)	65	65	33×22	33×22	1675	1095	650	698	653	661
		73	70	34×23	34×23						
20126	5 (39%)	62	64	33×21	33×21	1665	1100	646	896	671	673
		72	67	34×23	*36×25*						
19750	4 (18%)	60	59	32×21	32×21	1642	1589	637	645	764	757
		67	64	33×21	33×21						
19598	4 (11%)	57	58	33×20	33×20	1592	1578	617	609	654	653
		66	61	34×24	34×24						
9375	3 (6%)	54	53	31×20	*35×23*	1347	1087	519	510	571	568
		69	59	33×24	*36×24*						
19611	3 (9%)	53	57	30×21	30×21	1377	1363	531	527	610	613
		65	61	34×25	34×25						
9370	4 (17%)	60	58	30×22	30×22	1407	1396	543	517	560	556
		68	63	34×24	34×24						
20152	3 (8%)	56	48	30×21	30×21	1347	1339	519	515	581	579
		68	61	34×23	34×23						
Meteor-VRI	9 (95%)	97	98	35×24	35×24	1887	1057	703	710	773	753
		83	107	38×27	*40×28*						
PF-400	9 (96%)	95	95	34×24	*36×25*	1867	1029	695	719	785	777
		82	105	36×25	*39×27*						
Climax	9 (96%)	96	97	35×23	35×23	1913	1071	713	767	848	856
		84	106	37×36	*38×41*						
KQP-6121	8 (83%)	91	90	34×23	34×23	1794	1085	667	710	800	810
		80	101	36×25	36×25						
KQP-6173	8 (85%)	90	89	34×23	34×23	1809	1063	673	703	707	696
		82	102	36×26	*36×28*						
KQP-6185	9 (90%)	94	96	34×24	34×24	1951	1113	697	752	725	732
		81	103	37×27	37×27						
9800-5	9 (91%)	96	96	35×24	*37×23*	1930	1167	689	719	758	753
		82	105	36×26	*36×28*						
9800-10	8 (89%)	89	92	34×21	34×21	1860	1207	663	713	670	679
		81	101	36×25	36×25						
10649	8 (84%)	78	89	33×23	33×23	1725	1195	670	716	696	701
		79	100	36×24	36×24						
P1	8 (85%)	75	88	34×23	34×23	1762	1087	685	703	705	697
		80	95	35×26	*36×28*						
Premium	8 (86%)	77	85	35×21	35×21	1682	1027	653	711	784	793
		81	93	38×25	38×25						
Minimum		50	39	30×20	30×20	1151	1027	463	457	467	459
		63	55	30×20	30×20						
Maximum		97	98	35×24	37×25	1951	1589	713	896	848	856
		84	107	38×36	40×41						
Average		69.2	66.6	32×22	33×22	1537.3	1198.1	592.4	625.5	643.1	642.4
		72.5	76.3	34×24	35×25						
Standard Deviation		16.5	21.0	1.7×1.4	1.9×1.4	274.94	146.01	87.148	119.9	108.9	109.8
(SD)		7.2	20	1.7×2.8	2×3.7						
Coefficient of Variability		0.23	0.31	0.05×0.06	0.06×0.06	0.17	0.12	0.14	0.19	0.17	0.17
(CV)		0.10	0.26	0.05×0.11	0.06×0.15						

***** =The values for adaxial leaf surface are given in bold font, += The values for abaxial leaf surface are given in normal font, ^ α= values before Powdery mildew inoculation, > β= values after Powdery mildew inoculation

planted in pots containing silt loam (pH 7.6). The pots were placed in glass house and suggested cultural practices (Azmat *et al.,* 2011) were adapted for good crop husbandry. At 8th node stage all the genotypes were inoculated with powdery mildew inoculum in tween-20 and water-agar solution as described previously (Azmat *et al.,* 2012; Azmat and Khan, 2014). Control treatment without powdery mildew inoculation was also maintained.

***Measurement of number and size of stomata*:** Number and size of stomata were measured before and after inoculation; the data for control was also recorded. The leaf samples for stomatal observations were collected before and after powdery mildew inoculation (8th and 11th node stage, respectively). The leaf samples (five leaves) were taken from four plants of each genotype during peak photosynthetic hours (8-10 AM). Since pea leaves are of amphistomic type having stomata on both of the surfaces, therefore, observations on stomatal parameters were made for both abaxial and the adaxial surfaces. For stomatal studies impression technique (Hilu and Randall, 1984) was used. In this technique a film of clear nail polish was directly applied to the both surfaces of leaf. An excellent detailed image of epidermis was produced as the impression of stomata was left on nail polish film after drying. The leaf surface was cut in 2-2.5 cm^2 dimensions; clear scotch tape was used to transfer stomata included membrane to microscope slides. The observations on number and size of stomata were recorded in 3 different microscopic view fields using 40x10 magnifying lenses and ocular micro meter, respectively.

Measurement of chlorophyll and carotenoid*: *For chlorophyll and carotenoid concentration determination, the leaf samples were collected at above mentioned stages. The concentrations of carotenoid, Chlorophyll a and b per unit mass were measured spectrophotometrically using 80% (v/v) acetone for extraction, employing the equations of Lichtenhaler and Wellburn (1983).

***Disease severity*:** Data on disease severity was recorded 15 DAI (Days After Inoculation) as described previously (Azmat *et al*., 2012) to classify the genotypes as resistant and susceptible. The severity of disease was recorded on a 0–9 scale based on percentage of leaf area affected: 0 = no infection, 1 = <1%, 2 = 1%–5%, 3 = 5%–10%, 4 = 10%–20%, 5 = 20%–40%, 6 = 40%–60%, 7 = 60%–80%, 8 = 80%–90%, 9 = >90%.

***Statistical analysis*:** The experiment had three replications and all the values given here are the arithmetic means of all replications. Pearson correlations between traits were performed for susceptible and resistant genotypes separately and combined as well. A phenogram was constructed with Euclidian distance. Statistical analyses were carried out using SPSS 17 (SPSS, Chicago, I L), MVSP 3.1 (Kovach Computing Services, Anglesey, Wales), and Microsoft Excel (QI Macros).

RESULTS

The analysis of data has shown a range of variation among all the traits including disease score, number and size of stomata (adaxial and abaxial), concentration of total chlorophyll, chlorophyll a, b and carotenoid.

The disease score data based on percentage of leaf area affected has classified 30 genotypes in to two broader categories (16 resistant: 14 susceptible) with a disease score ranging from 0-9. Only two genotypes (It-96 and No.267) were highly resistant having no infection, while on the other extreme five highly susceptible genotypes (Meteor-VRI, PF-400, Climax, 9800-5, KQP-6185) had maximum disease score. The data for disease severity presented here also indicated that three genotypes (Acc. No. 18293, 20126 and 19616) that were previously selected as resistant (Azmat *et al*., 2012) have now emerged as susceptible to powdery mildew (Table 1).

There existed variability for number of stomata on both adaxial and abaxial surfaces before and after powdery mildew infection. Before powdery mildew infection number of stomata ranged from 50-97 and 63-84 with an average of 69.2 and 72.5 for adaxial and abaxial leaf surfaces, respectively. The size of stomata has shown comparatively less variability with a range of 30×20-35×24(µ) and 30×20-38×36(µ) on adaxial and abaxial surfaces of leaf respectively before powdery mildew infection (Table 1). The data has shown that after powdery mildew infection, on an average there was a decrease in number of stomata on adaxial leaf surface while an increase was observed on abaxial surface. The data has also shown a slight increase in the size of stomata on abaxial surface after disease incidence (Table 1; Fig. 1).

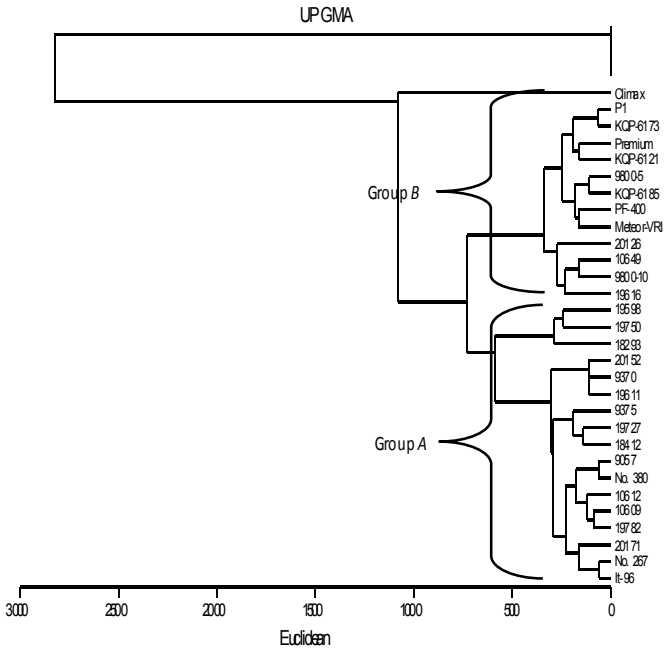

Figure 1. Euclidean distances and UPGMA based Phenogram showing independent grouping of resistant and susceptible pea genotypes.

Concentration of total chlorophyll (µg.g^{-1}) had significant variation both before and after powdery mildew infection and the same is the true for chlorophyll a (*Chl a*) and

Chlorophyll b (*Chl b*). The concentration of total chlorophyll ranged from 1614-2648 (μ =2130) and 1597-2234 (μ =1823) respectively before and after powdery mildew infection. The variation of *Chl a: Chl b* was also highly significant among the genotypes ranging from 2.48-2.73 with an average of 2.59 before powdery mildew infection. The data regarding *Chl a: Chl b* ratio after powdery mildew infection has shown highly significant decrease in this ratio ranging from 1.77-

Table 2. Correlation coefficients among different physiological traits in response to powdery mildew infection.

		SUB	SUA	SDB	SDA	SizUB	SizDB	SizUA	SizDA	ChlaB	ChlaA	ChlbB	ChlbA	CarB	CarA
	A	0.950**	0.983**	0.977**	0.981**	0.928**	0.731**	0.791**	0.709**	0.930**	-0.516**	0.912**	0.774**	0.844**	0.842**
PI	B	0.813**	0.915**	0.413	0.859**	0.582*	-0.132	0.397	0.047	0.729**	0.550*	0.746**	0.836**	0.691**	0.674**
	C	0.792**	0.970**	0.983**	0.967**	0.841**	0.668**	0.801**	0.586*	0.807**	-0.566*	0.747**	-0.579*	0.546*	0.506
			0.956**	0.945**	0.968**	0.924**	0.733**	0.801**	0.716**	0.909**	-0.449*	0.874**	0.730**	0.831**	0.826**
SUB			0.654**	0.646**	0.871**	0.759**	-0.438	0.452	-0.238	0.488	0.387	0.553*	0.716**	0.462	0.451
			0.883**	0.810**	0.886**	0.775**	0.657*	0.759**	0.588*	0.892**	-0.237	0.805**	-0.400	0.658*	0.607*
				0.964**	0.976**	0.930**	0.739**	0.820**	0.722**	0.952**	-0.421*	0.927**	0.783**	0.874**	0.869**
SUA				0.418	0.746**	0.509*	0.036	0.489	0.206	0.820**	0.586*	0.810**	0.810**	0.750**	0.737**
				0.964**	0.992**	0.859**	0.691**	0.809**	0.612*	0.878**	-0.442	0.817**	-0.479	0.583*	0.536*
					0.974**	0.931**	0.736**	0.836**	0.730**	0.893**	-0.561**	0.865**	0.732**	0.813**	0.808**
SDB					0.539*	0.548*	-0.376	0.704**	-0.099	0.220	-0.003	0.221	0.404	0.227	0.207
					0.963**	0.840**	0.733**	0.783**	0.666**	0.818**	-0.605*	0.755**	-0.490	0.593*	0.546*
						0.932**	0.748**	0.797**	0.720**	0.897**	-0.500**	0.862**	0.691**	0.812**	0.808**
SDA						0.604*	-0.464	0.284	-0.303	0.566*	0.480	0.608*	0.693**	0.583*	0.577*
						0.841**	0.663**	0.794**	0.580*	0.844**	-0.429	0.780**	-0.525	0.577*	0.530
							0.698**	0.867**	0.705**	0.878**	-0.483**	0.857**	0.710**	0.817**	0.803**
SizUB							-0.250	0.450	-0.115	0.385	0.325	0.472	0.634**	0.514*	0.499*
							0.607*	0.950**	0.596*	0.845**	-0.595*	0.861**	-0.405	0.562*	0.484
								0.586**	0.964**	0.661**	-0.459*	0.622**	0.479**	0.650**	0.650**
SizDB								-0.069	0.887**	-0.061	-0.089	-0.138	-0.139	-0.120	-0.124
								0.503	0.955**	0.677**	-0.419	0.792**	-0.109	0.734**	0.726**
									0.663**	0.738**	-0.566**	0.709**	0.616**	0.689**	0.668**
SizUA									0.346	0.194	-0.238	0.207	0.541*	0.227	0.197
									0.544*	0.798**	-0.595*	0.829**	-0.379	0.568*	0.486
										0.647**	-0.510**	0.620**	0.532**	0.649**	0.640**
SizDA										-0.066	-0.288	-0.125	0.125	-0.097	-0.118
										0.634*	-0.464	0.805**	0.034	0.719**	0.688**
											-0.253	0.986**	0.851**	0.919**	0.916**
ChlaB											0.881**	0.987**	0.674**	0.915**	0.914**
											-0.346	0.856**	-0.260	0.467	0.423
												-0.231	-0.294	-0.184	-0.181
ChlaA												0.878**	0.437	0.782**	0.795**
												-0.368	0.167	-0.406	-0.366
													0.889**	0.936**	0.933**
ChlbB													0.731**	0.924**	0.922**
													-0.238	0.623*	0.552*
														0.815**	0.815**
ChlbA														0.699**	0.670**
														-0.148	-0.120
CarB															0.998**
*. Correlation is significant at the 0.05 level (2-tailed).															0.997**
**. Correlation is significant at the 0.01 level (2-tailed).															0.987**

A= the value in row "A" are the combined coefficient of Pearson's correlation, B= the value in row "B" are the coefficient of Pearson's correlation for resistant genotypes only, C= the value in row "C" are the coefficient of Pearson's correlation for susceptible genotypes only.

The abbreviations used are PI= Percentage of leaf area infected, SUB = Number of adaxial stomata before powdery mildew, SUA= Number of adaxial stomata after powdery mildew, SDB= Number of abaxial stomata before powdery mildew, SDA= Number of abaxial stomata after powdery mildew, SizUB= Size of adaxial stomata before powdery mildew, SizDB= Size of abaxial stomata before powdery mildew, SizUA= size of adaxial stomata after powdery mildew, SizDA= size of abaxial stomata after powdery mildew, ChlaB= concentration of Chlorophyll a before powdery mildew, ChlaA= concentration of Chlorophyll a after powdery mildew, ChlbB= concentration of Chlorophyll b before powdery mildew, ChlbA= concentration of Chlorophyll b after powdery mildew, CarB= concentration of Carotene before powdery mildew, CarA= concentration of Carotene after powdery mildew.

2.24 and the average of this ratio after powdery mildew attack was 1.91 (Table 1).

As far as the concentration of carotenoid is concerned, it was also significantly different among all the genotypes. For carotenoid, it was observed that carotenoid is always in a tight ratio with *Chl b* ranging from 0.84-1.04 with an average of 0.94 regardless of the stage of powdery mildew infection (Table 1).

The coefficients of Pearson's correlation have shown that disease severity has highly significant positive correlation with all other traits except the concentration of *Chl a,* which has highly significant negative correlation with disease severity (Table 2).

The Euclidean phenogram constructed on the basis of all the traits have classified 30 genotypes into two distinct group *viz., Group A* (Resistant) and *Group B* (Susceptible; Fig. 1). The phenogram placed 16 genotypes in *Group A* and 14 in *Group B* (Fig. 1). Both the groups have shown clearly different response for all the parameters. The data values for each group were subjected to Pearson's correlation separately. In case of *Group A,* the coefficients of correlation indicated that all the traits had positive correlation with disease severity except number of stomata on abaxial leaf, size of stomata on both leaf surfaces after powdery mildew infection, and size of stomata on abaxial leaf before powdery mildew infection. In the susceptible group, the correlation coefficient for *Chl a* and *b* after powdery mildew infection had significant negative correlation with disease severity (Table 2). Both the groups of pea genotypes have shown significantly different values for each trait, while comparatively less variation was observed within each group (Fig. 1).

Both the groups showed different trends in response to powdery mildew infection. The response of number of adaxial and abaxial stomata to powdery mildew infection was significantly different among the groups. The number of stomata was decreased after powdery mildew infection in *Group A* by a factor of 1.13 and 1.1 for adaxial and abaxial leaf surfaces, respectively. The increasing trend was observed for *Group B* with factors 0.97 and 0.84, respectively for number of stomata on adaxial and abaxial leaf surfaces. For stomatal size, both groups have shown slight increasing trend after the incidence of powdery mildew. The concentration of *Chl a* decreased in both groups, but the decrease was more pronounced in *Group B* having a decrease factor of 1.6. The concentration of *Chl b* was increased in both groups by a factor of 0.98 and 0.91 for the resistant and susceptible genotypes respectively. The concentration of carotenoid remained unchanged before and after the incidence of disease (Fig. 1). Based on the findings of the current study, a comprehensive scale is devised to have precise selection of powdery mildew resistant and

Table 3. Physiological parameters based powdery mildew screening scale for pea (*Pisum sativum*).

Trait	Scale description	Formula	Ranking
Number of stomata (adaxial) before PM	1= ≤50-55, 2=56-61, 3=62-75, 4=76-90, 5=91-≥100	$DR = \frac{\sum Score\ all\ traits}{6}$	1=Resistant
Number of stomata (adaxial) after PM	1= ≤40-50, 2=51-59, 3=60-75, 4=76-90, 5=91-≥100		2=Moderately resistant
Number of stomata (abaxial) before PM	1=≥60-65, 2=66-70, 3=70-80, 4=80-90, 5=91-≥100		3=Moderately susceptible
Number of stomata (abaxial) after PM	1= ≤50-59, 2=60-64,3=65-75, 4=76-90, 5=91-≥100		4=Susceptible
↓*Chl a* B:*Chl a* A	1=≤1-1.06, 2=1.07-1.10, 3=1.11-1.3, 4=1.31-1.49, 5=1.50- ≥1.6		5=Highly susceptible
↑*Chl b* B:*Chl b* A	1=≥1-0.96, 2=0.97-0.93, 3=0.92-0.88, 4=0.87-0.84, 5=0.83- ≥0.79		

	PI	SUB	SUA	SDB	SDA	SizUB	SizDB	SizUA	SizDA	ChlaB	ChlaA	ChlbB	ChlbA	CarB	CarA
Min	**0***	**50**	**39**	**63**	**55**	**600**	**660**	**600**	**660**	**1151**	**1087**	**463**	**457**	**467**	**459**
	27**	62	60	68	65	640	748	640	748	1597	1027	646	698	653	661
Max	**18**	**61**	**59**	**70**	**64**	**704**	**850**	**805**	**864**	**1642**	**1589**	**637**	**649**	**764**	**757**
	96	97	98	84	107	840	1332	900	1558	1951	1375	713	896	848	856
Ave	**7.8**	**56.1**	**49.6**	**66.7**	**60.6**	**646.4**	**774.1**	**667.6**	**788.1**	**1312.6**	**1267.0**	**520.1**	**529.7**	**563.2**	**561.3**
	78.6	84.1	86.0	79.1	94.3	766.4	926.1	773.2	985.6	1794.1	1119.4	675.2	734.9	734.4	735.1
SD	**6.1**	**3.2**	**7.1**	**2.1**	**2.5**	**27.6**	**56.6**	**58.6**	**65.6**	**140.9**	**151.7**	**47.8**	**66.9**	**74.0**	**73.3**
	21.9	12.2	13.1	4.7	15.1	58.7	141.5	68.6	193.7	112.3	92.1	22.0	52.0	57.5	57.7
CV	**0.79**	**0.06**	**0.14**	**0.03**	**0.04**	**0.04**	**0.07**	**0.09**	**0.08**	**0.11**	**0.12**	**0.09**	**0.13**	**0.13**	**0.13**
	0.28	0.14	0.15	0.06	0.16	0.08	0.15	0.09	0.20	0.06	0.08	0.03	0.07	0.08	0.08
Factor	**Group**														
↓and↑	**A**	**↓1.13**		**↓1.1**		**↑0.97**		**↑0.98**		**↓1.04**		**↑0.98**		**1**	
	Group B	↑0.97		↑0.84		↑0.99		↑0.94		↓1.6		↑0.91		≈1	

*The values for resistant genotypes (Group A) are given in bold font, **The values for susceptible genotypes(Group B) are given in normal font

The abbreviations used are PI= Percentage of leaf area infected, SUB = Number of adaxial stomata before powdery mildew, SUA= Number of adaxial stomata after powdery mildew, SDB= Number of abaxial stomata before powdery mildew, SDA= Number of abaxial stomata after powdery mildew, SizUB= Size of adaxial stomata before powdery mildew, SizDB= Size of abaxial stomata before powdery mildew, SizUA= size of adaxial stomata after powdery mildew, SizDA= size of abaxial stomata after powdery mildew, ChlaB= concentration of Chlorophyll a before powdery mildew, ChlaA= concentration of Chlorophyll a after powdery mildew, ChlbB= concentration of Chlorophyll b before powdery mildew, ChlbA= concentration of Chlorophyll b after powdery mildew, CarB= concentration of Carotene before powdery mildew, CarA= concentration of Carotene after powdery mildew.

susceptible pea genotypes (Table 3).

DISCUSSION

Currently no high yielding powdery mildew resistant pea cultivar is commercially available. Efforts are being made for yield maximization but the incidence of powdery mildew disease is still the major bottleneck. The emergence of new pathotypes and the inability of host to pose resistance to all the pathotypes and isolates is the major reason for the failure, which can essentially be attributed to the inability to select the 'real' powdery mildew resistant plant source. The variation among resistant and susceptible pea genotypes regarding the concentration of total chlorophyll, *Chl a* and *b, Chl a: Chl b* and number of stomata on adaxial and abaxial leaf surface has provided a comprehensive screening method against powdery mildew. The results have indicated that the above mentioned traits have significant correlation with disease severity; hence, these traits can be used as selection criteria for powdery mildew screening.

It is an established fact that more the amount of chlorophyll in a plant more will be the photosynthetic activity and nutrients in the cell sap (Magyarosy *et al.,* 1976; Lichtenhaler and Wellburn, 1983). The obligate biotrophic fungi directly feed on the cell sap by penetrating haustoria. The fact mentioned above has provided a clue that more succulent genotypes having enhanced photosynthetic activities are comparatively more susceptible to powdery mildew pathogen. The results of the current study also supported that the powdery mildew resistant pea genotypes had significantly less concentration of total chlorophyll (Table 1). Moreover, comparatively a significant decrease in the concentration of total chlorophyll was also observed in susceptible genotypes after disease incidence (Fig.1).

A more pronounced substantial reduction in the *Chl a: Chl b* ratio was observed in susceptible pea genotypes, while an insignificant change in the ratio was observed for resistant genotypes. The reduction in *Chl a: Chl b* ratio was due to an increase in *Chl b* which represented an increase in light-harvesting chlorophyll (Scholes and Farrar, 1985). It is reported that the decrease in photosynthetic activity is due to fluctuations in the concentrations of total chlorophyll and *Chl a: Chl* b ratio (Sharkey, 1985; Scholes and Farrar, 1986). The damage to the electron transport chain and an inhibition of non-cyclic photophosphorylation are also the major cause of reduction in chlorophyll concentration and in turn the photosynthetic activity after powdery mildew infestation (Magyarosy *et al*., 1976; Sharkey, 1985; Scholes and Farrar, 1986).

It has been suggested that an increase in host and/or fungal respiration is partially responsible for the reductions in chlorophyll concentrations and photosynthesis in powdery mildew infected tissue (Farrar and Rayns, 1987). Stomata, the opening in the epidermis affect adaptation skills of plants by directing respiration, photosynthesis and transpiration (Brownlee, 2001). The alteration in the stomatal behavior in response to powdery mildew has already been reported (Ayres, 1976). Stomata fail to close completely leading to complete immobilization in a partly open position in the later stages of powdery mildew infection. Due to the change in stomatal behavior the rate of transpiration during dark increases and the presence of fungal mycelium on the leaf surface speed up the transpiration rate (Ayres, 1976; Gordon and Duniway, 1982). The rapid transpiration, enhanced respiration, reduction in photosynthetic activity and the concentration of chlorophyll are the typical symptomolgy of powdery mildew infected pea plants. The pea plants showing no change or comparatively less change to above physiological parameters are considered as powdery mildew resistant. The differential behavior of resistant and susceptible pea genotypes to these parameters has provided a new scale for screening against powdery mildew in pea (Table 3).

***Conclusion*:** Since, precise selection of powdery mildew resistant source is the key to develop high yielding and stable powdery mildew resistant cultivars in pea. Therefore, the screening method (based on physiological parameters) devised in this study would be helpful in making efficient, reliable and reproducible selections for powdery mildew resistance.

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