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SCREENING OF *PISUM SATIVUM* L. GERMPLASM AGAINST *ERYSIPHE PISI* SYD.

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Powdery mildew (*Erysiphe pisi* Syd.) significantly reduces the yield and quality of pea all over the world. Screening of a broad range of germplasm revealed three highly resistant genotypes (Fallon, PS99102238 and PS0010128); eleven (Shawnee, Lifter, Franklin, PS610152, PS810240, PS710048, PS610324, PS810191, CGN3273, CGN3272, and PS9910188) showed symptoms after inoculation but the infection was not severe and recovery was rapid. Powdery mildew caused 86% loss to the germplasm, and the severity of the disease was associated with various phases. The pathogen inhibits seed development in the pod. Severe natural infection is expected to eliminate susceptible germplasm, some of which may have valuable, unique characteristics. The screening data were used to explore the relationship between susceptible and resistant genotypes, and between genetic diversity and geographic patterns. Seed protein assays did not sort genotypes by geographic pattern or disease resistance. It is suggested to transfer genes conferring disease resistance and economic yield to one genotype.

Key words: Gel electrophoresis, pea, powdery mildew, seed protein.

INTRODUCTION

Pea (*Pisum sativum* L.) is an important source of vegetable protein (21–32% by weight) in large parts of the world. It is consumed as a green vegetable (whole pods or immature seed) in Asian countries and as dry seed in Europe, Australia, America and in Mediterranean regions. The major threat to its seed production is powdery mildew caused by *Erysiphe pisi*, for which resistance is available in germplasm (Sharma, 1995). Powdery mildew is the most widespread disease of *Pisum sativum* all over the world. An obligate parasite, its development depends on the photosynthetic status of the host; this pathogen cannot develop on photosynthetically inactive tissues (Caver and Jones 1988). The fungus is unique in that their haustoria penetrate only to epidermal cells devoid of chloroplasts; its successful development depends on the photosynthetic activity of underlying mesophyll cells. The pathogen causes up to 50% yield losses and reduces pod quality (Singh, 1987; Dixon, 1987). Air currents spread the fungus locally and over long distances, whereas rain controls the disease by washing off spores and making them burst instead of germinating (Hargedorn, 1991). Electrophoresis separates proteins by surface charge and protein size. SDS denatures polypeptide chains (as well as separate protein subunits in oligomers) and then surrounds individual polypeptide chains, giving each chain the same overall surface charge (Murphy et al., 1990). The present study was conducted to determine (1) whether powdery mildew affects initiation of flowering, (2) whether the disease affects vegetative maturity, (3) the effectiveness of artificial inoculation for screening, and (4) the usefulness of SDS-PAGE in determining disease status.

MATERIALS AND METHODS

In the greenhouse of the Institute of Agri-Biotechnology and Genetic Resources, National Agricultural Research Centre (Islamabad, Pakistan) 177 genotypes of *Pisum sativum* from 23 countries of six continents (Tab. 1) were planted during winter 2004–2005 (October 2004 to May 2005). Genotypes with CGN numbers were obtained from the Centre

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TABLE 1. Origin of 177 genotypes screened against powdery mildew

Origin	Frequency	Susceptible	Resistant	Highly Resistant
Albania	1	1	0	0
Australia	3	3	0	0
Canada	1	1	0	0
Czechoslovakia	2	2	0	0
Denmark	12	12	0	0
Ethiopia	12	12	0	0
France	2	2	0	0
Germany	3	3	0	0
Greece	4	4	0	0
India	3	3	0	0
Italy	2	2	0	0
Mexico	2	2	0	0
Netherlands	16	16	0	0
New Zealand	3	3	0	0
Norway	6	6	0	0
Pakistan	60	60	0	0
Peru	2	0	2	0
Poland	2	2	0	0
Russia	2	2	0	0
Sweden	14	14	0	0
Turkey	7	7	0	0
UK	3	3	0	0
USA	15	3	9	3

for Genetic Resources (Netherlands) genotypes with NGB numbers from the Nordic Gene Bank, and others (Lifter, Franklin, Joel, PS610152, PS610324, PS0010128, PS810240, PS10048, PS10191, Shawnee, Fallon, PS810765, PS9910188, PS99102238) from the United States Department of Agriculture (USDA). For screening, each genotype was planted in a row 4 m long, with 1 m inter-row and 10 cm intra-row spacing. Every second row was checked for the presence of fungus. As the fungus was present abundantly, infection occurred naturally. The data were recorded at different phases of the plant life cycle. Genotypes showing resistances under natural infestation were artificially inoculated with *Erysiphe pisi* conidia by tapping heavily infected plant parts over the leaves. In the resistant plants, infection was absent or localized in small patches (aspersoria) only on the foliage or the inoculated area. The severity of the disease was scored as 0%, 25% and ≥50%, and its effect on different phases as well as on different parameters was observed.

Of 177 genotypes, 102 that produced seed under high disease infection were analyzed for seed proteins by discontinuous slab electrophoresis on SDS-PAGE using 12.50% polyacrylamide gel measuring 7.5×9.5 cm^2. For extraction of proteins, single seeds were used for SDS-PAGE (Ghafoor et al., 2002). As all the accessions were pure lines, one seed from each genotype was analyzed for seed protein. To check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions. After electrophoresis, the gels were read by a direct photographic method (FA 500 EPI-Light UV gel documentation system) and depending upon the presence or absence of polypeptide bands, similarity indexes were calculated for all possible pairs of protein types. The scores were 1 for the presence and 0 for the absence of bands. All the analyses employed STATISTICA for Windows.

RESULTS

The disease caused 86% damage to the germplasm, and 19% of the genotypes could not enter the reproductive phase (Tab. 2). Of the 177 genotypes screened, 33 failed to initiate flowering due to the disease; these genotypes flowered in disease-free environments. Of the 144 genotypes that initiated flowering, 125 completed flowering and 24 produced viable seed, 3 of which were resistant, 11 tolerant and 10 susceptible. Disease severity varied with the plant life cycle phase. After 110 days of germination, 14 genotypes (PS99102238, PS9910188, PS810765, PS610324, PS610152, PS010128,

TABLE 2. Details of *Pisum sativum* germplasm screened against *Erysiphe pisi*

Vegetative Traits	Genotypes screened	Genotypes surviving	Survival percent	Resistant (no infection)	Tolerant (up to 25% infection)	Susceptible (more than 25% infection)
First flower initiation	177	144	81	14	15	115
Reached 100% flowering	144	125	87	14	8	103
Reached to vegetative maturity	125	24	19	3	11	10

Three highly resistant and 11 resistant genotypes are given in Table 3; susceptible genotypes produced viable seed were CGN3290, CGN3323, CGN3281, CGN3324, CGN3278, CGN3280, CGN3275, CGN3277, CGN3291 and CGN3327.

TABLE 3. Response of 14 resistant genotype to artificial inoculation by *Erysiphe pisi*

Genotype	Source	Observations after inoculation	Symptom appearance	% PM
PS810240	USDA	7 days	Symptom	25%
PS710048	USDA	7 days	Symptom	25%
PS610324	USDA	7 days	Symptom	25%
PS810191	USDA	7 days	Symptom	25%
PS9910188	USDA	7 days	Symptom	25%
Lifter	USDA	7 days	Normal	0%
		12 days	Symptom	25%
Franklin	USDA	7 days	Normal	0%
		12 days	Symptom	25%
CGN3273	Netherlands	7 days	Symptom	25%
		12 days	Symptom	25%
		At maturity	Normal	0%
CGN3272	Netherlands	7 days	Normal	0%
		12 days	Symptom	25%
		At maturity	Normal	0%
Shawnee	USDA	7 days	Normal	0%
		12 days	Symptom	25%
		At maturity	Normal	0%
PS610152	USDA	7 days	Normal	0%
		12 days	Normal	0%
		At maturity	Symptom	25%
Fallon	USDA	7 days	Normal	0%
		12 days	Normal	0%
		At maturity	Normal	0%
PS99102238	USDA	7 days	Normal	0%
		12 days	Normal	0%
		At maturity	Normal	0%
PS0010128	USDA	7 days	Normal	0%
		12 days	Normal	0%
		At maturity	Normal	0%

PS810240, PS710048, PS810191, CGN3272, CGN3273, Lifter, Franklin and Fallon) were resistant. Seven (PS99102238, PS9910188, PS810765, PS610152 A, PS010128, Shawnee and Fallon) were resistant after 150 days, and 11 (PS99102238, PS9910188, PS810765, PS610324, PS010128, CGN3272, CGN3273, Shawnee, Joel, Franklin and Fallon) were resistant at maturity. Disease intensity affected seed set. All the resistant genotypes produced fully filled pods with no empty locules.

To confirm disease resistance, resistant genotypes were inoculated artificially in another set of experiments under greenhouse conditions, and observations were recorded on daily. Fourteen resistant genotypes were inoculated till development of disease or otherwise. After a week of inoculation, genotypes PS9910188, PS810240, PS710048, PS610324, and PS810191 developed the disease, 4 genotypes (Lifter, Franklin, CGN3272, Shawnee) developed the disease after the second inoculation (12 days), and 3 genotypes (Fallon, PS99102238, PS0010128) were highly resistant (Tab. 3). CGN3272, CGN3273 and Shawnee showed epithesia and developed disease symptoms but, interestingly, the plants recovered resistance without the application of any fungicide. The 3 highly resistant genotypes were suggested for use in breeding programs to develop resistant cultivars.

On the basis of seed protein banding pattern, 26 bands were observed; 16 were polymorphic (Fig. 1). Cluster analysis sorted the genotypes into two major lineages (linkage distance 18.0). If the phylogenetic tree was observed at linkage distance 8.0, two lineages were further divided into seven clusters.

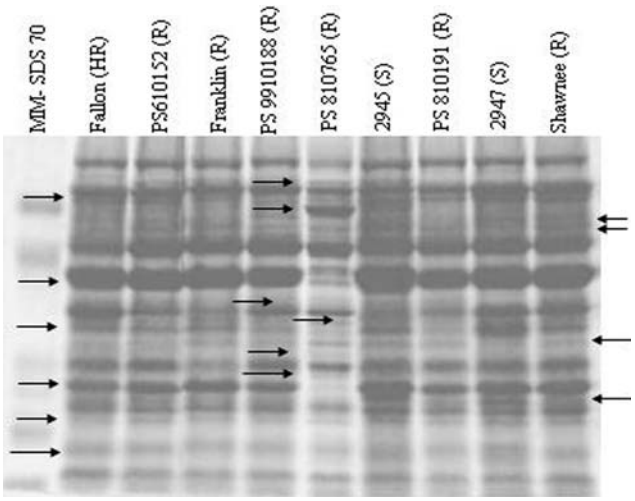

Fig 1. Seed protein profile in *Pisum sativum* screened against powdery mildew. Arrows indicate polymorphic bands. HR – highly resistant; R – resistant; S – susceptible to disease.

Lineage I was divided into two clusters (Fig. 2). Cluster 1 consisted of 8 genotypes (CGN3294, CGN3285, CGN3289, CGN3293, CGN3292, CGN3287, CGN3284, CGN3283) all of them originated from Pakistan. Cluster 2 comprised 13 genotypes (CGN3280, CGN3278, CGN3282, CGN3277, CGN3267, CGN3255, CGN3248, CGN3254, CGN3299, CGN3272, CGN3303, CGN3295, CGN2916); of these, 7 were from Pakistan, 4 from Ethiopia, and one each from Peru and the Netherlands. Cluster 3 consisted of 12 genotypes, all from Pakistan except Fallon from the U.S.A. Cluster 4 consisted of mixed genotypes from 9 countries.

Clusters 5 and 6 comprised 30 genotypes having one highly resistant (PS99102238), 4 resistant (PS9910188, CGN3273, PS610152 and PS610324) and 25 susceptible genotypes. Cluster 7 contained 9 genotypes from the U.S.A., 3 from Pakistan, 2 from the Netherlands, and one each from New Zealand, Sweden, Turkey and Denmark. In cluster 7 were one highly resistant (PS010128), 8 resistant escaping (PS810240, PS810765, Shawnee, PS710048, PS810191, Lifter, Joel and Franklin) and 9 susceptible genotypes.

DISCUSSION

Powdery mildew causes qualitative as well as quantitative losses to crops (Ahmad et al., 2001). Conventional breeding can be an option for develop-

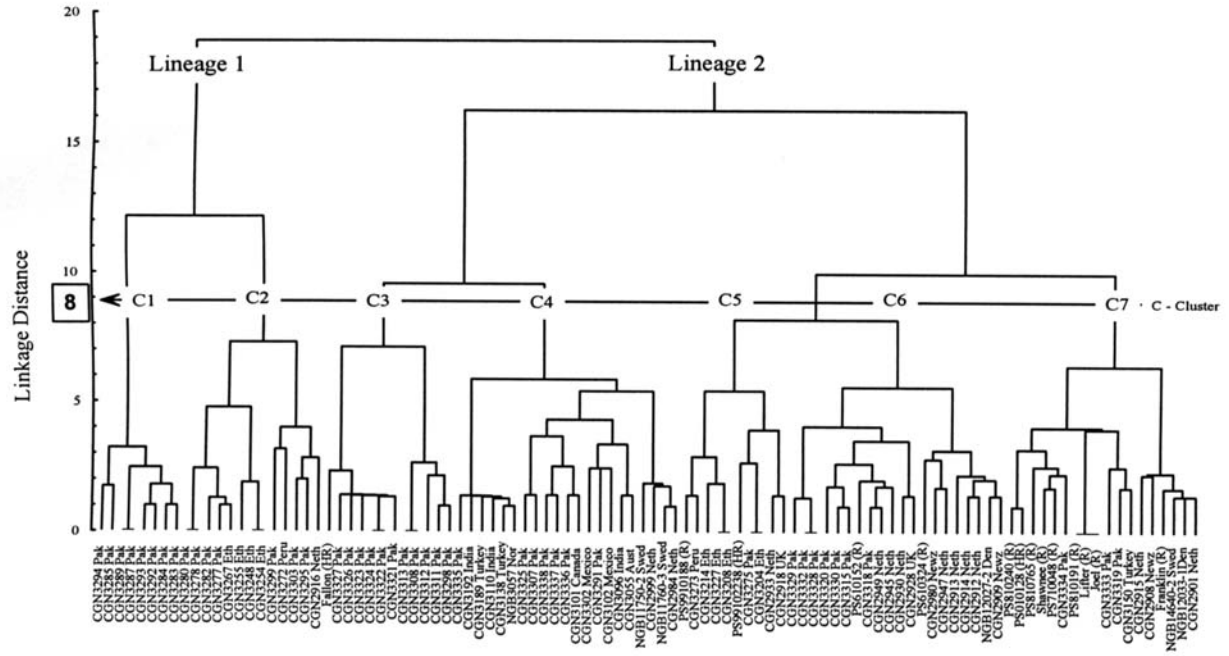

Fig. 2. Phylogenetic tree for total seed protein profile of *Pisum sativum* L. HR and R represent highly resistant and resistant genotypes, respectively; the others were susceptible to powdery mildew. Country codes: Aust – Australia; Den – Denmark; Eth – Ethiopia; Neth – Netherlands; Newz – New Zealand; Nor – Norway; Pak – Pakistan; Swed – Sweden.

ment of resistant cultivars if resistant sources are available. Among the resistant genotypes, three were highly resistant, and these could be used in breeding programs for development of disease-resistant and high-yielding germplasm. Recovery after localized infection, as observed in three genotypes, is rather attributable to development of photosynthetically inactive tissue at maturity (Caver and Jones, 1988; Ghafoor et al., 2005). SDS-PAGE is a particularly reliable method for assessment, because storage proteins are largely independent of environmental fluctuations (Gepts, 1989; Murphy et al., 1990). Although there was some variation in the banding pattern of total seed protein, it was not correlated with the geographic pattern or disease reaction. Genotypes showing highly individual banding profiles can be selected as genetically divergent (Nisar, 2004). Seed protein profiles did not sort resistant genotypes into one cluster; they were intermingled with others. The proximity of genotypes had no close relationship with *Erysiphe pisi* sensitivity. Although a single recessive gene for powdery mildew resistance has been reported (Sharma, 2003; Janila and Sharma, 2004), SDS-PAGE did not reflect the product of this gene, so other biochemical techniques such as 2D-electrophoresis or DNA markers are needed. The relationship between seed protein banding and disease status may be of use broadly, but we observed no such linkage. The screening procedure was conducted under controlled conditions, with optimum levels of inoculum applied, hence our report on screening is based on plants with actual genetic resistance to the fungus.

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