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Morphological Characterization and Selection for High Yielding and Powdery Mildew Resistant Pea (*Pisum sativum*) Lines

(Pencirian Morfologi dan Pemilihan Titisan Kacang Pea (*Pisum sativum*) untuk Hasil Tinggi dan Tahan Kulapuk Berdebu)

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ABSTRACT

Twenty lines of Pisum sativum *particularly developed for high yield and resistant to powdery mildew were evaluated along with two parents (Falloner and 11760-3ER) and two checks (Climex and a local cultivar) with the objectives to determine morphological characterization, yield potential and resistance to powdery mildew. On the basis of one way cluster, the 24 lines were mainly grouped into four clusters, especially on the vegetative and yield contributing traits. It was observed that the tall and high yielding lines were grouped in cluster-III while the dwarf and high yielding lines were grouped in cluster-IV. Analysis of variance (ANOVA) showed significant difference (*p*<0.05) in the yield of 24 pea lines. The average grain yield of the 24 pea lines ranged from 22.87 to 102.54 g. The highest grain yield was produced by PL-4 (102.54 g plant-1) followed by PL-5 (82.14 g plant-1). Of the 24 pea lines, two lines (PL-4 and PL-5) were highly resistant to powdery mildew disease. Therefore, the newly developed PL-4 and PL-5 lines were high yielding and highly resistant. Among the 19 morphological traits, six (Eigenvalue* >*1.0) contributed more than 80% variability among the materials.*

Keywords: Morphological characterization; pea lines; powdery mildew; statistical analysis; yield potential

ABSTRAK

Dua puluh titisan Pisum savitum *dibangunkan untuk mendapatkan hasil yang tinggi dan tahan terhadap kulapuk berdebu telah dinilai bersama dua induknya (*Fallon*er dan 11760-3ER) dan dua pengesah (*Climex *dan kultivar tempatan) dengan objektif untuk mengenal pasti pencirian morfologi, potensi hasilan dan daya tahan terhadap kulapuk berdebu. Berasaskan kelompok sehala, 24 titisan telah dibahagikan kepada empat kelompok terutamanya ciri vegetatif dan penyumbang hasil. Dapat diperhatikan, titisan yang panjang dengan hasil yang tinggi telah dikumpulkan dalam kelompok-III, manakala titisan yang kerdil dengan hasil yang tinggi termasuk dalam kelompok-IV. Analisis varians (ANOVA) menunjukkan perbezaan yang ketara (*p*<0.05) dalam hasil kesemua 24 titisan kacang pea. Purata hasil bijian daripada 24 titisan kacang pea adalah antara 22.87 kepada 102.54 g. Hasil tertinggi bijian telah keluaran daripada PL-4 (102.54 g tumbuhan-1), diikuti PL-5 (82.14 g tumbuhan-1). Daripada 24 titisan kacang pea, dua titisan (PL-4 dan PL-5) mempunyai daya tahan terhadap penyakit kulapuk berdebu yang tinggi. Maka, titisan PL-4 dan PL-5 yang baru dibangunkan adalah berhasilan tinggi dan mempunyai berdaya tahan yang tinggi. Antara 19 ciri morfologi, enam (nilai* Eigen*>1.0) telah menyumbangkan lebih daripada 80% perbezaan dalam kesemua bahan yang terhasil.*

Kata kunci: Analisis statistik; kulapuk berdebu; pencirian morfologi; potensi penghasilan; titisan kacang pea

INTRODUCTION

Pea (*Pisum sativum*) is a member of family Papilionaceae (Muhammad et al. 2009). It is cultivated worldwide on an area of 6.33 million ha with annual production of 10 million metric tons (FAOSTAT 2012). It is cultivated under a wide range of agro-ecological zones of Pakistan on 10,000 ha for green pods with a production of $82,000$ tons year¹ (Khan et al. 2013).

The economic importance of pea is mainly due to the chemical constituents of its seeds. It is a rich source of proteins (10.9%), carbohydrates (60.7%), fats (1.4%) and an important source of nutrition for human beings (Tzitzikas et al. 2006) as well as fodder for animals (Nemecek et al. 2008). The primary goal of plant breeding is to increase

yield potential to meet the increasing food demand. New varieties with improved agronomic traits have been the major contributing factor to increase yield potential (Nisar et al. 2008). Morphological characterization is the first step in the description, classification and identification of high yielding and disease resistant germplasm in the diverse gene-pool of plant species (Muhammad et al. 2009; Naghavi & Jahansouz 2005). Generally, the significance of morphological and genetic characterization in crops has been highlighted by both plant breeders and geneticists for further crop improvement (Able et al. 2007). Morphological techniques including cluster analysis, principal components analysis (PCA) and correlation coefficient have been successfully used to classify and

measure the pattern of genetic characterization as reported in blackgram (Ghafoor & Arshad 2008), pea (Ali et al. 2007), lentil (Sultana & Ghafoor 2006) and chickpea (Naghavi & Jahansouz 2005).

The grain yield of pea is adversely affected by powdery mildew caused by *Erysiphe pisi*. Powdery mildew is a serious problem that decreases number of nodes, plant height, number of pods, number of seeds, grain yield and plant biomass (Ghafoor & McPhee 2012). Generally the disease is controlled by applying chemicals that harm human health, therefore eco-friendly method is to develop new varieties with genetic resistance (Cao et al. 2011). In view of the cost-effective solution for powdery mildew disease, there is a need to develop high yielding and powdery mildew resistant varieties (Ghafoor & McPhee 2012). Limited information is available on genetic improvement of pea in Pakistan due to low priority in research and little attention has been paid by legume scientists (Ghafoor et al. 2005). Keeping in view the importance of pea, the present work was focused to evaluate hybrid pea lines for important agronomic traits and select high yielding and powdery mildew resistant elite lines for further utilization.

MATERIALS AND METHODS

PLANT MATERIALS

Twenty advanced lines of *P. sativum* were developed through single seed descent method from the cross Falloner/11760-3ER made in 2004 at National Agricultural Research Center (NARC) as reported by Nisar and Ghafoor (2009) . Another four lines, two were parents (Fallon^{er}) (PL-23) and 11760-3ER (PL-24)), Climex (PL-22) and local cultivar (PL-21), were used as checks for comparative analysis and interpretation.

EXPERIMENT AND DATA RECORDING

Evaluations of the 24 pea lines were carried out for three consecutive years 2011-2012, 2012-2013 and 2013-2014. The plants were planted in November and harvested in April at University of Malakand, Khyber Pakhtunkhwa, Pakistan located at coordinates N 34° 40" 318' and E72° 03" 753' at altitude 726 m. The 24 lines were grown in a layout of complete randomized block design with three replicates in row plots as suggested by Hanaa and Ali (2011). The length of each plot was 18 m with a width of 3 m. Each line was planted in a row plot with 75 cm row to row in distance and 10 cm plant to plant in distance (Nisar et al. 2011).

After seed germination, 10 plants were tagged randomly within each line for morphological data scoring as well as powdery mildew scoring. A total of 19 morphological traits were recorded using modified descriptors of Seed Certification Department, Pakistan (Tajammal & Naz 2009). Powdery mildew disease was synthetically inoculated by spreading the contaminated

debris of previous year of pea crop. Disease was recorded using a 0-5 scale (Anonymous 2010), where 0 is plants with no infection (highly resistant); 1 is 1.0-10.0% of leaf area infection (resistant); 2 is 10.1-25.0% of leaf area infection (moderately resistant); 3 is 25.1-50.0% leaf area infection (moderately susceptible), 4 is 50.1-75.0% leaf area infection (susceptible) and 5 is the rating for plants with >75% of leaf area infection (highly susceptible).

STATISTICAL ANALYSIS

Different statistical tools were employed for the analysis of morphological traits. The average data of 10 randomly selected plants were statistically analyzed (Keneni et al. 2005). One way cluster analysis of morphological traits was carried out by using the statistical software PC-ORD ver. 5 (McCune & Grace 2005). The objective of the analysis was to determine genetic linkage among the 24 pea lines. In addition to the cluster analysis, K-mean value was calculated to determine genetic variation among different clusters and Pearson's correlation coefficients were determined among the various traits by using SPSS software ver. 18.0 (Keneni et al. 2005). Principal component analysis (PCA) was also performed for morphological traits using Minitab (Minitab Inc. 2008). Analysis of variance (ANOVA) was performed for yield performance to assess significant differences among the yield of different pea lines. Basic statistics including mean and standard error were also calculated using software Statistica ver. 8.

RESULTS

MORPHOLOGICAL CHARACTERIZATION

Morphological trait analysis of germplasm is utmost important for selection of genotypes for a particular agronomic trait. One way cluster analysis (OWCA) was carried out to estimate the genetic distance/linkage among the 24 pea lines based on agro-economical traits. Four clusters were observed at 80% dissimilarity based on agroeconomical traits (Figure 1). It was observed that the lines within the same cluster were closely linked and exhibited genetic similarity for agronomic traits. In the present study, it was observed that the lines grouped in the cluster-I were tall (average height 137.9 cm) but low yielding (average yield 37.09 g plant⁻¹). While the lines of cluster-II were dwarf (71.875 cm) and low yielding (43.15 g) , the lines in cluster-III were tall (153.74 cm) and high yielding (73.25 g). On the other hand, cluster-IV contained dwarf (82.99 cm) lines with high yielding ability (61.91 g).

The *K-Mean* values were estimated for the grouping 24 lines to show trait homology. Based on the four clusters, *K-Mean* values showed varying degrees of genetic diversity and levels of trait contribution across the clusters (Table 1). The pea lines in the cluster-III were better for pod number, pod weight and seed yield plants-1, whereas the lines having lower values for these traits were in the cluster-I. The clusters-II and IV constituted pea lines with

FIGURE 1. One-way cluster analysis based on the 19 agro-economical traits of the 24 pea lines

TABLE 1. *K-Mean* clusters analysis and cluster average values for 19 quantitative traits

	Cluster-I	$Cluster-II$	$Cluster-III$	Cluster-IV	Cluster average $&$ St. Er
Days to germination	11.25	$12**$	11.00	$10.83*$	11.27 ± 0.26
Days to logging	$37.63*$	57.75**	41.00	48.33	$46.18 + 4.46$
Node at which branches start	$3.18**$	2.52	2.75	$2.43*$	2.72 ± 0.17
Branches per plant	6.14	$3.63*$	5.92	$6.68**$	5.59 ± 0.67
Leaflet per leaf	3.75	$3.00*$	5.33	$5.67**$	4.44 ± 0.64
Internode length (cm)	6.86	3.75	$7.50**$	$3.35*$	5.37 ± 1.06
Days to flowering	118.95	$114.56*$	118.99	$120.66**$	118.29 ± 1.31
Node at which pod start	14.38**	$10.5*$	13.33	11.00	12.30 ± 0.93
Plant height	137.96	71.87*	153.75**	83.00	111.65 ± 20.14
Plant life spin	$181*$	181.75	181.50	185.83**	182.65 ± 1.06
Plant biomass	91.17*	103.05	$216.13**$	182.67	148.25 ± 30.40
Pod length	7.38	$7.23*$	7.89**	7.56	7.52 ± 0.14
Pod width	$1.50**$	1.31	1.48	$1.29*$	1.40 ± 0.05
Pod per plant	35.59*	38.91	$61.41**$	56.31	48.05 ± 6.36
Pod weight per plant	$50.57*$	67.18	95.74**	80.81	73.58±9.63
Seeds per Pod	5.58	$5.51*$	$6.36**$	6.06	5.88 ± 0.20
100 seed weight	$30.44**$	23.42*	31.17	27.54	28.14 ± 1.76
Grain yield per plant	37.09*	43.15	$73.25**$	61.91	53.85 ± 8.35
Harvest index	40.67	42.08**	34.48	34.29*	37.88±2.04

*Lowest value and ** Highest value

intermediate pod number plants-1, pod weight plants-1 and seed yield plant⁻¹.

All the traits were subjected to principal component analysis (PCA) for estimation of weight contribution of each trait and to evaluate the total level of genetic diversity. Four components gave Eigenvalues >1.0, thus they were important in consideration of genetic variability amongst all the lines. Four components (PC1-PC4) contributed 70% genetic variability (Table 2). The PC1 explained 26.9% of the total variability. Leaflet leaf⁻¹, biomass, pod length, pod plant-1, pod weight plant-1, seeds pod-1 and grain yield plant-1 were the variables with the largest positive loadings. However, germination duration with negatively loading

was observed for this component. The PC2 explained 24.9% of the total contribution toward variability. Node at which branch start, internode length, node at which pod start, plant height, pod width and 100 seed weight were the variables with high positive loading whilst days to logging was negatively loaded. The third component (PC3) contributed 10.7% of variability and was more related to vegetative phase but negatively for days to flowering. The PC4 explained 7.5% of the total variance and related to high positive loadings of days to germination, branches plant-1 and leaflet leaf-1 along with negative loadings for node at which branches start, days to flowering, pod length and harvest index.

	PC1	PC2	PC ₃	PC4
Days to germination	$-0.229**$	-0.092	$0.223*$	$0.365*$
Days to logging	-0.060	$-0.386**$	-0.058	0.036
Node at which branches start	-0.125	$0.247*$	-0.002	$-0.192**$
Branches per plant	0.185	0.082	-0.109	$0.428*$
Leaflet per leaf	$0.209*$	0.008	$0.221*$	$0.466*$
Internode length (cm)	-0.003	0.389*	$0.276*$	-0.011
Days to flowering	0.169	0.162	$-0.409**$	$-0.189**$
Node at which pod start	-0.100	$0.392*$	-0.026	0.052
Plant height	0.060	$0.414*$	0.140	0.028
Plant life spin	0.134	-0.095	$-0.447**$	0.017
Plant biomass	$0.400*$	0.002	-0.088	0.154
Pod length	$0.251*$	0.076	0.077	$-0.399**$
Pod width	0.015	$0.344*$	0.096	0.034
Pod per plant	$0.413*$	-0.053	0.056	0.002
Pod weight per plant	$0.390*$	-0.102	0.100	-0.095
Seeds per pod	$0.253*$	-0.025	$0.461*$	-0.051
100 seed weight	0.104	$0.295*$	$-0.255**$	-0.050
Grain yield per plant	$0.402*$	-0.084	0.080	-0.072
Harvest index	-0.092	-0.180	$0.321*$	$-0.430**$
Eigenvalue	5.1056	4.7350	2.0278	1.4281
Percent variability	0.269	0.249	0.107	0.075
Cumulative variability	0.269	0.518	0.625	0.700

TABLE 2. Principal component analysis (PCA) of 19 traits among pea lines, Eigen values, percentage variability explained by first four components

*High positive loading and **High negative loading

Inter-relationships based on Parson's correlation coefficients are presented in Table 3. Grain yield showed high and significant (*p*≤0.01) correlation with biomass $(r=0.85)$, pods plant⁻¹($r=0.89$), pod weight plant⁻¹($r=0.95$) and seeds pod⁻¹ ($r=0.53$). 100 seeds weight was positively and significantly correlated with days to flowering (*r*=0.53). However, it had significantly negative relationship with days to lodging $(r=0.56)$. Seeds pod⁻¹ had high and positive correlation with pods plant-1 (*r*=0.54) and pod weight plant⁻¹ ($r=0.53$). Pod weight plant⁻¹ was positively and significantly correlated with plant biomass (*r*=0.83) and pods plant⁻¹ ($r=0.90$). Similarly pod plant⁻¹ was highly and significantly correlated with plant biomass (*r*=0.88).

The selected edible pea lines were evaluated for quantitative traits for three consecutive years, i.e. 2011- 12, 2012-2013 and 2013-14 (sowing in November and harvested in April) in order to identify the best candidate cultivar(s) with high yield production. The grain yield of the 24 pea lines ranged from 22.87 to 102.54 g. Grain yields of the pea lines for the three years are presented in the Table 4. The genotype PL-4 produced the maximum seed yield $(102.54 \text{ g plant}^{-1})$ followed by PL-5 (82.14 g) plant⁻¹), whereas the lowest seed yield $(22.87 \text{ g plant}^{-1})$ was produced by PL-14.

The ANOVA was calculated for yield, in order to find significant differences in the yield of the 24 edible pea lines. The average three years grain yield of PL-1, PL-2, PL-3, PL-7, PL-8, PL-11, PL-12, PL-13, PL-14, PL-15, PL-16, PL-19, PL-21 and PL-23 was significantly ($p < 0.05$) lower

as compared to the average three years grain yield of PL 22 (Climex). On the other hand, the average three-year yield of PL-4, PL-5, PL-9 and PL-10 showed significantly (*p*<0.05) higher yield as compared to PL 22. However, average three-year grain yield of PL-6, PL-17, PL-18, PL-20 and PL-24 showed no significant difference in yield as compared to PL22 (Table 4).

DISEASE SCREENING

The 24 edible pea lines were screened against powdery mildew disease caused by *Erysiphe pisi* for the three consecutive. The symptoms of the disease started to appear in the last week of March and reached its peak by the end of April. Hence forward, it was found that out of the 24 pea lines, three lines (PL-4, PL-5 and PL-23) were highly resistant (DSS-0), seven (PL-1, PL-2, PL-3, PL-6, PL-11, PL-16 and PL-19) were rated as resistant (DSS-1), three (PL-10, PL-12 and PL-13) were moderately resistant (DSS-2), four (PL-7, PL-14, PL-17 and PL-18) were moderately susceptible (DSS-3), five (PL-8, PL-9, PL-15, PL-20 and PL-21) were susceptible (DSS-4) and two lines (PL-22 and PL-24) were highly susceptible (DSS-5) (Table 5).

DISCUSSION

Knowledge on genetic characterization for a given population is of interest not only for their protection and registration but also for practical applications

 $.20112013$ TABLE 3. A matrix of correlation coefficients (r) of the 19 agro-morphological traits of pea evaluated during 2011-2013 J. ۔۔۔
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'Correlation is significant at the 0.05 level and **Correlation is high significant at the 0.01 level *Correlation is significant at the 0.05 level and **Correlation is high significant at the 0.01 level

DG=Days to germination, DL=Days to logging, NBS=Node at which branches start, BP=Branches per plant, L/L=Leaftet per leaft, Il=Internode length, DF=Days to flowering, NPS=Node at which pod start, PH=Plant height, PLS=Plant DG=Days to gegmination, DL=Days to logging, NBS=Node at which branches start, B/P=Branches per plant, L/L=Leaflet per leaf, IL=Internode length, DF=Days to flowering, NPS=Node at which pod start, PH=Plant height, PLS=Plant PB=Plant biomass, PL=Pod length, PW=Pod width, P/P=Pod per plant, PW/P=Pod weight per plant, S/P=Seeds per pod, 100SW=100 seed weight, GY/P=Grain yield per plant and HI=Harvest index. * The mean value of morphological traits of three years (2011-2013) were computed for estimation of r-value

	Year 2011 Yield weight (g)	2012 Yield weight (g)	2013 Yield weight (g)	Average yield $&$ S.E.
PL1	31.79	28.71	32.25	30.92^b ±1.11
PL ₂	38.01	34.43	37.22	$36.55^{\rm b}$ ±1.09
PL ₃	46.14	43.71	48.93	46.26° ±1.51
PL 4 Candidate	99.06	101.68	106.87	$102.54^a \pm 2.29$
PL 5 Candidate	86.20	75.09	85.14	$82.14a + 3.54$
PL 6	59.67	67.54	56.21	61.14 ± 3.35
PL7	50.18	47.26	54.33	$50.59^{\rm b}$ +2.05
PL 8	48.68	38.78	42.73	$43.40^{\rm b}$ ±2.88
PL 9	74.43	72.89	77.16	$74.8^{\text{a}} \pm 1.25$
PL 10	73.85	79.84	66.85	$73.51^{\circ}+3.75$
PL 11	54.22	48.29	51.35	51.29° ±1.71
PL 12	40.18	39.14	45.36	41.56^b +1.92
PL 13	48.06	38.43	44.74	43.74° +2.82
PL 14	25.89	22.23	20.51	$22.87^{\rm b}$ ±1.59
PL 15	31.65	43.11	37.33	$37.36^b \pm 3.31$
PL 16	41.30	33.45	36.68	$37.14^b \pm 2.28$
PL 17	63.28	69.56	57.42	$63.42 + 3.51$
PL 18	60.85	66.43	57.14	61.47 ± 2.70
PL 19	43.26	48.14	44.70	45.37° ±1.45
PL 20	67.62	65.95	59.98	64.52 ± 2.32
PL 21 (L. Cultivar)	44.32	34.64	41.23	$40.06^{\rm b}$ ±2.85
PL 22 (Climex)	64.03	57.13	67.08	62.74 ± 2.95
PL 23 (Fallon ^{er})	45.59	39.31	48.75	$44.55^{\rm b}$ ±2.77
PL 24 (11760-3ER)	66.87	56.61	64.31	62.60 ± 3.08

TABLE 4. Disease response and ANOVA for yield performance of the 24 pea lines

average yield values with superscript "a" show significantly high yield (p <0.05) and values with superscript "b" show significantly low yield $(p<0.05)$ as compared to PL22 by one way ANOVA, S.E. = standard error

DSS-disease severity scale; PL- pea lines; *F*- frequency; %- percentage

 $0 =$ highly resistant, $1 =$ resistant, $2 =$ moderately resistant, $3 =$ moderately susceptible, $4 =$ susceptible and $5 =$ highly susceptible

including conservation of genetic resources and for breeding purposes, to predict the ability to combine, or fast verification of breeding material (Ouji et al. 2011). Agronomic performance is the only criterion for farmers that includes better traits for pod weight, grain yield and harvest index, and selected lines exhibiting higher agronomic performance are likely be adopted by farmers for general cultivation (Twari & Lavanya 2012).

Four components as observed in PCA contributed more than 70% of variability amongst all the 24 pea lines and the importance of this technique has been reported appreciably for germplasm management (Maji & Shaibu 2012). Morphological characterization is the first choice for classification and documentation and it is still considered important in plant species either alone or in combination with biochemical and molecular assays (Bouhadida et al. 2013). However, interaction between genotype and environment complicates the evaluation process, especially on quantitative traits (Vieira et al. 2007).

Without understanding of character association in complex biological commodities, genetic improvement could not precisely be addressed (Esiyok et al. 2011). Thus, the correlation statistics were conducted that indicated positive correlation of seed yield with pods plant¹ and pod weight and similar results have been reported in pea (Kumar et al. 2013; Sharma et al. 2007; Singh et al. 2007) and in soybean (Balcha 2014). Seed yield potential of the grain crops depends upon harvest index. Although pea is mainly consumed as fresh pods for vegetable use, with the development of powdery mildew resistant material, we expect that pea has the potential for dry seed production in Pakistan. The importance of harvest index for ensuring

seed yield has been investigated in the present study, the lines PL-4 and PL-5 were superior for seed yield performance, hence genotype-environment interaction of these lines will be investigated for recommendation for general cultivation.

Powdery mildew disease adversely affects the yield potential, causing 86% loss to pea germplasm growing in different parts of the world (Nisar et al. 2006). Furthermore, spore release can cause breathing and allergic reactions in farm workers (Ek et al. 2005). In order to control disease, farmers often use chemical agents for controlling the disease, which may cause environmental pollution (Bhattacharjee & Dey 2014). Therefore, genetic based resistance for pathogenic diseases is the best option for crop breeding (Fondevilla & Rubiales 2012). The selected pea lines were also evaluated for powdery mildew disease in order to select powdery mildew resistant lines for documentation and identification of superior lines for general cultivation. The screening against powdery mildew had been conducted by various researchers (Nisar et al. 2006; Shahid et al. 2010) using different methods, including artificial inoculation is the most effective and robust (Nisar et al.2006; Sillero et al. 2006). Under heavy inoculums and conducive conditions for powdery mildew, the lines PL-4 and PL-5 were highly resistant indicating the worth of these lines for powdery mildew resistance.

CONCLUSION

In the present study, the 24 pea lines were evaluated morphologically and a considerable level of genetic diversity was observed indicating the potential of selection for promising cultivars. PL-4 and PL-5 lines were found to be high yielding and powdery mildew resistant homozygous and they could be selected as elite lines for general cultivation. These lines are to be evaluated under wider range of agro-climatic condition in the province as well as throughout Pakistan to understand geneenvironment interaction for spot selection.

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