

Research Report

Inheritance Studies of *Pisum sativum* F₁, F₂ and F₃ Generation Based Morphological Traits and Selection of High Yielding Powdery Mildew Resistant Lines

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Abstract Aim of this study was to determine the inheritance and linkage of six contrasting traits in *Pisum sativum* F₁ and F₂ generation, and to select high yielding powdery mildew resistant lines in F₃ generation. Two isogenic lines Falloner and 11760-3ER were selected crossed. All the traits in F₂ segregants, showed 3:1 ratio, which was fit for goodness by χ^2 ($p>0.07$) method and indicates monogenic inheritance. Anthocyanin pigmentation linked with flower colour with likely hood ratio 44.31 and with seed colour at 34.91. While tendrils type linked with number of leaflet per leaf at likely hood ratio 33.21. All the three linked qualitative traits were highly significantly correlated at $P<0.00$. None of the qualitative trait was linked to powdery mildew disease. In F₃ generation four yields contributing quantitative traits were studied. Among the quantitative traits, total pod weight (49.77%) and number of seeds/pod (20.01%) showed maximum level of coefficient of variation. In correlation studies, total pod weight pods was high significantly positively correlated with seed weight, pod width, pod length, number of seed/pod. Similarly, pod length was highly positively significantly correlated with number of seeds/pod. Based on yield contributing traits and powdery mildew resistance three lines GN070140-2, GN070143-1 GN070140-0 was selected, which could use as cultivar in future.

Keywords Peas, Inheritance, *er-1* gene, Analysis, Novel three lines

The genus *Pisum* contains two species, *P. sativum* and *P. fulvum*, both with $2n=14$ chromosomes. The dried pea contains 10.9% protein, 1.4% fat, 60.7% carbohydrate, 1.4% crude fiber, and 2.7% ash (Tzitzikas et al., 2006). Powdery mildew, caused by *Erysiphe pisi*, is the most wide spread disease of *Pisum sativum* all-over the world. Powdery mildew causes the crop losses reaching as high as 25%~86%.

Genetic marker provide an attractive alternative to desirable traits selection, making breeding process more efficient and less resource demanding. Once a genetic marker that is closely linked to the desirable traits has been identified, marker assisted (MAS) can be practiced at early stage of plant development, thus avoiding selection through traits exposure (Rakshit et al., 2001). MAS can be useful not only for qualitative traits controlled by a single gene but also for quantitative traits (Lande and Thompson, 1990). However, the complex architecture of quantitative traits may limit the efficiency

of MAS for each trait (Ek et al., 2005). Morphological markers are limited in nature, but their association with any particular trait is very beneficial as it neither required sophisticated equipments nor complicated procedures. Monogenic or oligogenic morphological markers are until recently scientific plant classification and novel gene identification was based exclusively on morphological traits (Fondevilla et al., 2007) some of which may serve as genetic marker suitable for plant germplasm management (Stanton et al., 1994).

In Pakistan pea is cultivated under a wide range of agro-ecological zones. It is cultivated during winter in plains of Pakistan and during summer in highlands (Habib and Zamin, 2003). During 2005-2006, the crop was grown over an area of 90.3 thousands ha with 52.4 thousand tones production of dry pea, but the average/hectare yield is very low as compared with its potential and yield obtained in many other countries (Anonymous, 2007). Little attention has been given to

varietals improvement of peas and is used as a marginal crop for cultivation in Pakistan (Bashir and Arshad 2002). Among the grain legumes, dry peas (*Pisum sativum*) ranks first in production in Europe, while in Pakistan the production of peas Kgs/ha is almost static from the past 15 years (Anonymous, 2007). Development of new pea cultivars for Pakistan pulse growers is a critical component in the effort to ensure that the cost of production remains competitive and the crop retain its high quality. Breeding, mutation, genetic engineering, indigenous and exotic germplasm evaluation and conservation are very essential to develop high yielding genotype and to broaden the gene pool (Ghafoor et al., 2003).

Due to the contradictory reports on powdery mildew resistance gene inheritance and divesting effect of the pathogen on yield, a study was made to confirm the exact nature of the powdery mildew resistant gene and select high yielding powdery mildew resistance lines. The efforts were extended to select morphological markers to be used in Marker Assisted Selection (MAS) breeding program.

1 Materials and Methods

1.1 Screening of germplasm

A total of 177 genotypes obtained from different part of the globe were planted in the screen house at the Institute of Agri-Biotechnology and Genetic Resources, National Agricultural Research Centre, Islamabad, Pakistan during winter 2004–2005. Optimum conditions for fungus growth were provided during the experiment, the genotypes showed resistances under natural infection were artificially inoculated with *Erysiphe pisi*

conidia by tapping heavily infected plant part over leaves. Two isogenic lines falloner (powdery mildew resistant) and 11 760–3ER (powdery mildew susceptible) having six morphological contrasting traits i.e. anthocyanin pigmentation, flower colour, pod colour, tendrill type, leaf type and seed texture were selected and cross was made to ensure the inheritance and association (linkages) of the traits (Table 1).

1.2 Inheritance qualitative traits

F₁ seeds (♀ Fallon×11760–3 ♂) were sown in the screen house of IABGR, NARC during 2005–2006 to get the F₂ seeds. To determine the inheritance of powdery mildew and its association with other qualitative traits, parents along with F₁ and F₂ seeds were sown in the screen house of IABGR, NARC during 2006–2007. Optimum conditions were provided for the growth of fungus *Erysiphe pisi*; however, the resistant plants were artificially inoculated with conidia of the fungus by dusting from the leaves. The data were recorded on 46 F₂ segregates on individual plant basis.

1.3 Inheritance of quantitative traits

To select high yielding powdery mildew resistant genotype, check varieties DASAN was also sown for comparison. The F₂ generation was sown during 2007–2008 in the same condition and recommended procedures used for F₁ generation to get F₃ generation. In F₃ generation, four yield contributing quantitative traits; total pod weight, twenty seed weight, pod length and number of seeds pods were studied in 97 plants.

1.4 Data analysis

The inheritance and association of various qualitative and quantitative traits in F₂ and F₃ generation were

Table 1 Six contrasting traits among the selected parents of *Pisum sativum*

Serial No.	Contrasting traits	Parents	
		Fallon (Parent – ♀)	11760–3 (Parent– ♂)
1	Anthocyanin pigmentation	Pigmentation absent	Pigmentation present
2	Flower colour	Creamy	Purple
3	Pod colour	Green	Purple
4	Tendrill	Bushy	Normal
5	Number of leaflets	Leafless	Leaflet present
6	Seed texture	Smooth	Rough
7	Disease	Resistant	Susceptible

subjected to different statistical analysis. The data scored on qualitative traits (F₂) were analyzed through chi-square test and likelihood ratios, while that of quantitative traits (F₃) were subjected to frequency distribution, coefficient of variation and correlation coefficient. Based on total pod weight and powdery mildew sensitivity high yielding powdery mildew resistant lines were selected.

2 Results

2.1 Inheritance of powdery mildew disease

In the present investigation plant response to *Erysiphe pisi* appeared to be under the control of two alleles (susceptible and resistant) of single gene. All the F₁ were susceptible indicating the recessive expression of the *er-1* gene. Similarly, F₁ generation showed that anthocyanin pigmentation present, flower colour purple, pod colour purple, tendrils normal, leaf type leaflet present, seed texture rough were the dominant alleles over anthocyanin pigmentation absent, flower colour creamy, pod colour green, tendrils bushy, leaf type

leafless and seed texture smooth (Table 2).

2.2 Analysis F₂ generation

For F₂ population, the observed value count of susceptible was 33/46 and resistant 13/46. Chi square (χ^2) for the expected value 3:1 ratio was calculated (0.2) and was fit for goodness by χ^2 ($p > 0.65$), indicated the monogenic inheritance for powdery mildew disease (Table 2). Anthocyanin pigmentation, flower colour, pod colour, tendrils type, leaf type and seed texture showed 3:1 ratio, which was fit for goodness by χ^2 ($p > 0.07$) method and also indicates monogenic inheritance (Table 2).

In likelihood ratio analysis, out of seven qualitative contrasting traits; anthocyanin pigmentation high significantly linked with ($p < 0.00$) with flower colour, seed colour and pod colour with likelihood ratios 44.31, 34.91 and 10.31, respectively. It was also calculated that, tendrils type and flower colour was high significantly linked ($p < 0.00$) with number of leaflet/leaf and pod colour at likelihood ratios 33.21 and 10.32, respectively (Table 3).

Table 2 Segregation for eight qualitative contrasting traits in F₂ generation of in *Pisum sativum*

Serial No.	Contrasting traits	Dominates×Recessive	Observed value		Expected Value	χ^2	P at 5%
1	Anthocyanin pigmentation	PP×pp	PP, Pp	pp	3:1	3.22	0.07
			Present	Absent			
			29	17			
2	Flower colour	CC×cc	CC, Cc	cc	3:1	3.22	0.07
			Purple	Creamy			
			29	17			
3	Pod colour	GG×gg	GG, Gg	gg	3:1	1.24	0.26
			Green	Purple			
			31	14			
4	Tendrils type	NN×nn	NN, Nn	nn	3:1	1.41	0.21
			Normal	Bushy			
			37	8			
5	Leaf type	LL×ll	LL, Ll	ll	3:1	2.64	0.10
			Leaf present	Leafless			
			39	7			
6	Seed texture	SS×ss	SS, Ss	ss	3:1	0.20	0.64
			Rough	Smooth			
			33	13			
7	Sensitivity to powdery mildew <i>er-1</i> gene	RR×rr	RR, Rr	rr	3:1	0.20	0.64
			Susceptible	Resistant			
			33	13			

Table 3 Association for five combinations of contrasting traits in F₂ population of *Pisum sativum*

Serial No.	Morphological traits		Likely hood ratio	Sig.
1	Anthocyanin pigmentation	Flower colour	44.31	0.00**
2	Anthocyanin pigmentation	Seed colour	34.91	0.00**
3	Tendrill type	No-leaflet/leaf	33.21	0.00**
4	Flower colour	Pod colour	10.32	0.00**
5	Anthocyanin pigmentation	Pod colour	10.31	0.00**

Note: *: Significant (Sig.) at P ≤ 0.05 level; **: high significant (Sig.) at P ≤ 0.01 level

2.3 Analysis F₃ generation

In coefficient of variation out of five quantitative traits total pod weight (49.77%) and number of seeds/pod (20%) showed maximum level of coefficient of variation (Table 4). Frequency distributions for the traits were calculated to classify F₃ generation into different categories (Figure 1). It indicated that 18.55% of the populations were having ≥ 31.94~47.9 g total pods weight and 10.30% of the segregants were ranged in ≥ 7.33~9.17 g twenty seeds weight. Similarly, 11.34% of the total population having ≥ 8.83~11.05 cm pod length and 19.58% were ranged ≥ 5.5~6.8 number of seeds

Table 4 Correlation coefficient of F₃ generation of *Pisum sativum* (L.) based on quantitative traits

Traits	Total pod weight	Seed weight	Pod length
Total pod weight	1		
Seed weight	0.27**	1	
Pod length	0.24**	0.15*	1
Number of seeds/pod	0.23**	0.05	0.35**

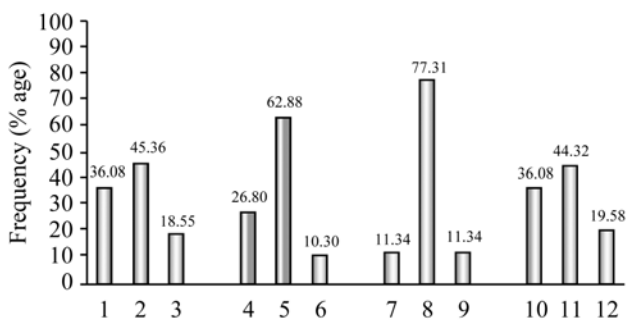


Figure 1 Percentage frequency distribution of five quantitative traits in F₃ generation of *Pisum sativum* (L.)

Note: 1~3: Total pod weight (g); 1 ≥ 5.08~5.96; 2 ≥ 15.97~31.93; 3 ≥ 31.94~47.90; 4~6: 20 seeds weight (g); 4 ≥ 3.66~5.49; 5 ≥ 5.50~7.32; 6 ≥ 7.33~9.17; 7~9: Pod length (cm); 7 ≥ 4.41~6.61; 8 ≥ 6.61~8.82; 9 ≥ 8.83~11.00; 10~12: Number of seed/pod; 10 ≥ 2.8~4.2; 11 ≥ 4.3~5.4; 12 ≥ 5.5~6.3

pod weight (Figure 1).

In correlation coefficient total pod weight highly significantly positively correlated with twenty seed weight (r=0.27), pod length (r=0.24) and number of seeds/pod (r=0.23). Likewise, pod length was also highly significantly positively correlated with number of seeds/pod (Table 4). Based on quantitative evaluation in comparison with checks variety DASAN, three lines GN070140-2, GN070143-1 and GN070140 were selected as high yielding powdery mildew resistant lines (Figure 2). In figure 2, vertical block indicates contribution of the each line and check variety with correspondence to four quantitative traits. Intercept displayed average contribution of each line and check variety to quantitative traits. Intercept clearly grouped the selected three lines into G- I and cultivars (DASAN) into G- II (Figure 2).

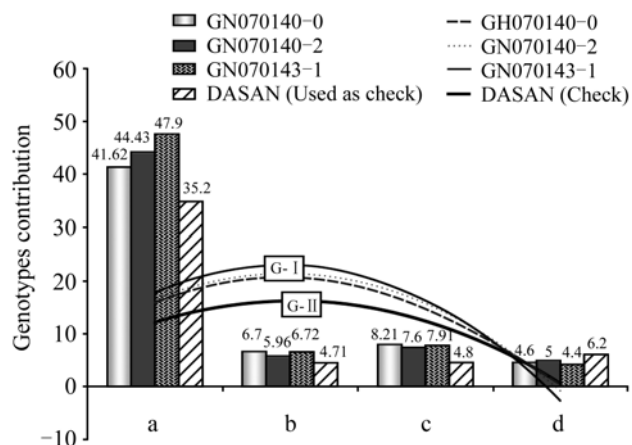


Figure 2 Comparison of three high yielding lines with check variety (DASAN) in F₃ population of *Pisum sativum* (L.)

Note: Vertical block indicates contribution of the lines and check variety with correspondence to four quantitative traits. Set intercept displayed average contribution of each line and check variety to four quantitative traits. G- I group grouped novel lines and G- II encircle only check variety DASAN; a: Total pod weight/plant; b: Seed weight; c: Pod length; d: No seed/pod

3 Discussion

Conventional plant breeding methods have been effective in bringing about improvement but efforts are still being made to develop more efficient breeding methods to overcome specific problem (Fondevilla et al., 2005). Contradictory reports regarding the inheritance of powdery mildew in pea have been reported by different authors. Resistance to powdery mildew in pea was first reported as monogenic recessive with the gene symbol *er* by Harland (1948) as digenic i.e., *er1* and *er2* (Heringa et al., 1969; Kumar and Singh, 1981). Fondevilla (2007) identified a new gene *er3* for resistance to powdery mildew in *Pisum fulvum* and wild relative of pea based on morphological traits. While in the present study an ideal condition was provided to investigate the exact nature of powdery mildew sensitivity. It was concluded that all F₁ generation showed the susceptible phenotype indicating the dominance of susceptible allele over the resistant allele. The F₂ generation for powdery mildew disease segregated with 3:1 ratio, which was fit for goodness by χ^2 test. It was concluded that *er-1* gene is controlled by two homozygous recessive alleles. All the remaining qualitative traits also showed monogenic nature of inheritance.

In the present study it was found that anthocyanin pigmentation linked with flower colour and seed colour, while tendril type linked with number of leaflet per leaf. None of the qualitative trait was linked to powdery mildew disease. The finding is needed to be explored through biochemical and molecular markers. The gene expression and traits interaction can also be explored using serial analysis of gene expression (SAGE) as developed by Velculescu et al. (1995) is a high-throughput method to determine the absolute abundance of every transcript in a population of cells (Matsumura et al. 2005; 2006).

Breeding is the option to bring heterogeneity and vigor in the germplasm reported by Ghafoor et al. (2003). In the present study F₃ generation calculates maximum level of coefficient of variation for total pod weight and number of seeds/pod. It is widely recognized that variation is the basis of improvement. Correlation is the measure of the degree to which variables

vary together or a measure of intensity of association. Guler et al. (2001) studied linear relationship among yield and yield component. Similarly high significantly positive correlation was observed in all studied yield contributing traits except between number of seeds/pod and seed weight. Based on yield contributing quantitative traits evaluation in comparison with checks variety (DASAN), three lines GN070140-2, GN070143-1 and GN070140 were selected as high yielding powdery mildew resistant lines. The novel high yield powdery mildew resistant lines is super lines which can replace DASAN due to high yield and negative response to powdery mildew disease caused by *Erysiphe pisi*. The lines are most suitable for cultivation in Pakistan. These lines were systematically conserved in Bannu GenBank, Department of Biotechnology, University of Science and Technology Bannu, NWFP, Pakistan.

From the present study it is concluded that powdery mildew resistance gene (*er-1* gene) is under the control of homozygous recessive allele. It also indicated that anthocyanin pigmentation linked with flower, pod and seed colour. While tendril type and flower colour linked with number of leaflet per leaf and pod colour respectively. Similarly it was investigated that none of the qualitative trait linked with powdery mildew disease. All these linked traits should be used in MAS breeding and gene mapping. Three novel high yielding lines were selected and should be used as cultivars, needed further improvement. To enhance the food value and for economic benefits the breeder selects high yielding and resistant cultivars and discard the high yielding susceptible or low yielding resistant lines. It is suggested that, conserve all the population, especially high yielding susceptible or low yielding resistant lines in the GenBank for molecular characterizations and other biotechnological uses.

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