Indian Journal of Agricultural Sciences **82** (2): 190–2, February 2012

A quantitative analysis of rust (*Uromyces fabae*) resistance in pea (*Pisum sativum*) using RILs*

A K SINGH[1], R RAI[2], C P SRIVASTAVA[3], B D SINGH[4] C KUSHWAHA[5] and R CHAND[6]

Banaras Hindu University, Varanasi, Uttar Pradesh 221 005

Received : 13 December 2010; Revised accepted: 16 September 2011

Key words: AUDPC, Average degree of dominance, Heritability, Pea, *Uromyces fabae*

Pea rust caused by *Uromyces fabae* (Pers. de-Bary) is a major disease of pea (*Pisum sativum* L.) worldwide. Under epidemic conditions it causes substantial yield losses, particularly in warm humid conditions (Chand *et al*. 2006, Kushwaha *et al.* 2010). *U. fabae* is an autoecious fungus and incidence of rust at early growth stages may result in complete failure of the crop. The genetics of rust resistance in pea is still unclear, and workers have reported a single dominant gene (Tyagi and Srivastava 1999), a single oligogene (Vijayalakshmi *et al.* 2005) showing partial dominance along with some minor genes, and involvement of one to two major genes (Singh and Ram 2001). One of the best possible ways to stabilize the productivity of pea crop is to grow rust-resistant varieties. Therefore, enhancement of rust resistance in pea cultivars is a major challenge, which needs to be addressed on priority. A more clear understanding of the genetics of rust resistance in pea will facilitate efforts to develop resistant cultivars by facilitating selection for rust resistance in the segregating generations. The objective of the present study was to estimate heritability, average degree of dominance and the number of genes controlling rust resistance in pea.

The experimental material consisted of 136 recombinant inbred lines (RILs) developed from the cross HUVP 1 (susceptible) × FC 1 (resistant). Seeds from random single F_2 plants were harvested separately in 2000-01, and 15 seeds from each of the 250 F_2 plants were composited and planted in bulk in the *rabi* season of 2001–02, and F_4, F_5 and F_6 generations were raised following single seed descent method. The materials were grown at the Agriculture Research Farm, Banaras Hindu University, Varanasi, India. $F_{6:7}$, $F_{6:8}$ and $F_{6:9}$ RILs were evaluated under polyhouse conditions during the *rabi* seasons of 2005–06, 2006–07 and 2007–08, respectively. Seeds were sown in pots filled with garden soil. Upon germination the population was maintained at 5 plants/pot, and one pot represented one replication. The experiment was laid out as per randomized block design with two replications.

Single pustule inoculum of pea rust was multiplied on a highly susceptible genotype HFP 4 grown in polyhouse. Twenty severely infected leaves of HFP 4 were soaked in 500 ml water for 2 hr and the spore suspension was filtered through cheesecloth. Water was added to adjust the spore density to 10^4 spores/ml (using a haemocytometer) before inoculating the test genotypes using a hand sprayer. The sprinkler fitted at top of the polyhouse was used for misting to maintain appropriate humidity for pathogenesis (Chand *et al*. 2004). After inoculation, five plants of each RIL and twenty-five plants of parental genotypes in each replication were tagged at 10^{th} nodal leaf before the expected appearance of the disease in the polyhouse, and data on disease severity were recorded three times at intervals of one week.

Disease severity for each line was visually estimated on a 0-9 scale (Sokhi *et al.* 1984) after initiation of rust infection on susceptible check, HUVP 1. Homogeneity of error variances was tested before combining the data over years by using Bartlett's test. Heritability (h^2) in broad-sense was calculated according to Hallauer and Miranda (1981). The estimates of Average degree of dominance (ADD) were obtained according to Mather and Jinks (1971) and the minimum number of effective genes controlling pea rust resistance was estimated by the method of Wright (1968):

$$N = R^2 / 4.27\acute{o}^2g$$

Where, N is the estimated minimum number of effective genes segregating, $\acute{o}^2g$ is the genetic variance of F_6 lines

*Short note

Based on a part of Ph D thesis of the first author submitted to BHU, Varanasi in 2010

[1]Ph D student (e mail: aksingh25@gmail.com); [3]Professor (e mail: cpsgenetics@gmail.com), Department of Genetics and Plant Breeding; [5]Post Doctoral Fellow (chandakushwaha@yahoo.co.in); [6]Professor (e mail: rc_vns@yahoo.co.in), Department of Mycology and Plant Pathology; Institute of Agricultural Sciences; [2]Ph D student (e mail: rashmi28rai@gmail.com); [4]Professor (e mail: brahmadsingh@gmail.com), School of Biotechnology; Faculty of Science

(measured as phenotypic variance of F_6 lines – ½ (variance of parent 1 + variance of parent 2), and R is the estimated genotypic range.

The average disease severity over three years was 20.5% in the resistant parent FC 1 and 62.3% in the susceptible parent HUVP 1. Similarly, the average AUDPC values for rust over three years were 322.5 for FC 1 and 824.4 for HUVP 1, which indicated that the parental lines displayed contrasting phenotypes for pea rust severity (Table 1). In combined analysis, disease severity ranged from 14.9 to 66.8% with a mean value of 40.5% in the RILs, and AUDPC values ranged from 214.3 to 925.5 with a mean value of 549.5, indicating a large phenotypic variation in the RIL population (Table 1). The above features of rust in the RIL population suggested quantitative nature of disease resistance (Tekeoglu *et al.* 2000). Vijayalakshmi *et al* (2005) also suggested quantitative nature of pea rust resistance in terms of number of pustules/cm^2 leaf area.

Heritability (h^2) estimates for disease severity were 0.93, 0.94 and 0.95 during 2005–06, 2006–07 and 2007–08, respectively, and 0.90 in the combined analysis. Similarly, heritability estimates for AUDPC were 0.95, 0.97 and 0.95 in 2005–06, 2006–07 and 2007–08, respectively, and 0.93 in the combined analysis (Table 1). The heritability estimates for disease severity corresponded closely with those for AUDPC of the respective years. High heritability of disease severity and AUDPC suggested that selection for pea rust resistance can be made under polyhouse conditions using either disease severity or AUDPC as disease reaction indicator (Negussie *et al.* 2005).

Rust severity of the F_1 derived from the cross HUVP 1 × FC 1 in 2006–07 was 36.2%, those of resistant and susceptible parents were 17.5% and 59.5%, respectively. Similarly, the mean AUDPC of the F_1 was 445.5, whereas those of the resistant and susceptible parents were 233.2 and 724.6, respectively. Pairwise comparison among FC 1, HUVP 1 and the F_1 showed that the F_1 was significantly different from the parents, but not from the mid-parent value (Table 1). Average degree of dominance (ADD) for resistance to *U. fabae* was 0.11 and 0.14 for disease severity and AUDPC, respectively, indicating that the genes controlling resistance to *U. fabae* exhibited a low degree of incomplete dominance.

Estimates of minimum number of effective genes conferring resistance to pea rust varied from two to three using three different methods of estimation (Table 2). When parental difference was used as a measure of genotypic range, the estimated number of genes varied from 1.15 to 1.76, but when phenotypic differences among the RILs were used as a measure of genotypic range, the estimated number of genes varied from 2.23 to 2.64. However, when phenotypic difference of RILs multiplied by heritability was used as a measure of genotypic range, the estimated number of effective genes varied from 1.93 to 2.48 (Table 2).

Table 1 Mean, range, heritability and average degree of dominance (ADD) for disease severity (%) and AUDPC for pea rust (*U. fabae*) in RILs derived from the cross HUVP 1 × FC 1 in pea

Genotype	Disease severity (%)				AUDPC			
	2005–06	2006–07	2007–08	Combined	2005–06	2006–07	2007–08	Combined
HUVP 1	70.60 ± 0.70	59.50 ± 0.45	61.20 ± 0.55	62.30 ± 0.51	975.50 ± 10.88	724.60 ± 4.75	757.50 ± 4.47	824.40 ± 4.96
FC 1	23.30 ± 0.47	17.50 ± 0.36	18.50 ± 0.40	20.50 ± 0.37	365.80 ± 7.21	233.20 ± 3.31	275.50 ± 3.48	322.50 ± 3.79
F_1		36.20 ± 0.65		36.20 ± 0.65		445.50 ± 3.75		445.50 ± 3.75
RILs (mean)	47.02 ± 1.92	36.53 ± 1.64	37.92 ± 1.66	40.50 ± 1.68	648.10 ± 24.10	478.50 ± 20.92	522.0 ± 19.45	549.53 ± 21.28
RILs (range)	19.25 – 74.25	11.25 – 61.85	12.75 – 65.0	14.92 – 66.83	287.50 – 1087.75	145.62 – 842.50	210.0 – 897.50	214.37 – 925.58
Heritability (h^2)	0.93	0.94	0.95	0.90	0.95	0.97	0.95	0.93
ADD		0.11		0.11		0.14		0.14

[a]F_1 was not evaluated during 2005–06 and 2007–08

Table 2 Estimated number of genes segregating for disease severity (%) and AUDPC for pea rust (*U. fabae*) in RILs derived from the cross HUVP 1 × FC 1 in pea

Character	Year of estimate	Number of genes			
		Method I[a]	Method II[b]	Average[c]	Method III[d]
Disease severity (%)	2005–06	1.65	2.23	1.94	1.93
	2006–07	1.76	2.54	2.15	2.28
	2007–08	1.74	2.61	2.18	2.36
	Combined	1.57	2.41	2.00	1.97
AUDPC	2005–06	1.33	2.28	1.81	2.06
	2006–07	1.31	2.64	1.98	2.48
	2007–08	1.16	2.36	1.76	2.16
	Combined	1.15	2.30	1.73	2.01

[a]method I, R, Phenotypic range of parents: [b]method II; R, phenotypic range of RILs: [c]average, average of gene number estimated by method I and method II: [d]method III, R, phenotypic range of RILs × heritability

The method of using parental differences as a measure of genotypic range often underestimates the number of genes, while, the method using phenotypic difference of the progeny lines as genotypic range tends to overestimate the number of genes. The method employing the phenotypic range of the progeny lines accounting for environmental influence (multiplying the range by the heritability of the RILs) gives a better estimate of genotypic range. The average of the number of genes estimated by the first two methods corresponded closely to that from the third method; it seems that 2-3 genes govern rust resistance in the cross HUVP 1 × FC 1. QTL mapping for rust resistance using SSR markers has, in fact, identified 3 QTLs in this cross (Rai *et al*. 2011). The lack of standardized methodology, including control on inoculum and environmental conditions, limits comparisons of results from different studies (Takeoglu *et al.* 2000). In addition, pathotype variation is a potent factor in such studies, and this aspect needs to be carefully controlled in inheritance studies.

Thus it may be concluded that in pea rust resistance is controlled by 2–3 additive genes. The heritability of partial resistance to pea rust is quite high (> 0.90) so that selection for resistance would be effective, especially when carried out under relatively controlled conditions using either disease severity or AUDPC as disease reaction estimates.

SUMMARY

In a quantitative analysis of pea rust resistance using a RIL population, heritability estimates for disease severity and AUDPC were found to be 0.90 and 0.93, respectively. High heritability indicated that selection for pea rust resistance can be made under polyhouse conditions using either disease severity or AUDPC as disease reaction estimators. Average degree of dominance (ADD) for resistance to *U. fabae* was 0.11 and 0.14 for disease severity and AUDPC, respectively, indicating that the genes controlling *U. fabae* resistance exhibited a low degree of incomplete dominance. Estimates of the minimum number of effective genes conferring resistance to pea rust, using three different methods of estimation, varied from two to three.

REFERENCES

Chand R, Srivastava C P and Kushwaha C. 2004. Screening technique for pea (*Pisum sativum*) genotypes against rust disease (*Uromyces fabae*). *Indian Journal of Agricultural Sciences* **74**: 166–7.

Chand R, Srivastava C P, Singh B D and Sarode S B. 2006. Identification and characterization of slow rusting components in pea (*Pisum sativum* L.). *Genetic Resources and Crop Evolution* **53**: 219–24.

Hallauer A R and Miranda J B. 1981. *Quantitative Genetics in Maize Breeding*. Iowa State University Press, Ames.

Kushwaha C, Chand R, Srivastava CP, Singh AK, Rai R and Singh BD 2010. Importance of aecial cups/pustule for selection for slow rusting in pea (*Pisum sativum*) against *Uromyces fabae*. *Indian Journal of Agricultural Sciences.* **80** (10): 933–6.

Mather K, and Jinks J L. 1971. *Biometrical Genetics: The Study of Continuous Variation*. Cornell University Press, Ithaca, New York.

Negussie T, Pretorius Z A and Bender C M. 2005. Components of rust resistance in lentil. *Euphytica* **142**: 55–64.

Rai R, Singh AK, Singh BD, Joshi AK, Chand R and Srivastava CP. 2011. Molecular mapping for resistance to pea rust caused by *Uromyces fabae* (Pers.) de-Bary. *Theoretical and Applied Genetics* **123**: 803–13.

Singh R and Ram H. 2001. Inheritance of powdery mildew and rust resistance in pea. *Annals of Biology* **15**: 13–6.

Sokhi S S, Singh S J and Munshi G D. 1984. Parameters of rating pea varieties/lines against rust. *Indian Phytopathology* **37**: 252–5.

Tekeoglu M, Santra D K, Kaiser W J and Muehlbauer F J. 2000. Ascochyta blight resistance inheritance in three chickpea recombinant inbred line populations. *Crop Science* **40**: 1251–6.

Tyagi M K and Srivastava CP. 1999. Inheritance of powdery mildew and rust resistance in pea. *Annals of Biology* **15**: 13–6.

Vijayalakshmi S, Yadav K, Kushwaha C, Sarode S B, Srivastava C P, Chand R and Singh B D. 2005. Identification of RAPD markers linked to the rust (*Uromyces fabae*) resistance gene in pea (*Pisum sativum*.). *Euphytica* **144**: 265–74.

Wright S. 1968. *Evolution and Genetics of Populations,* Vol. I, pp 469 Genetic and Biometric Foundations. University of Chicago Press, Chicago, USA.