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RESEARCH ARTICLE

Evaluation of French Bean Genotypes with Slow Rusting Components Against *Uromyces appendiculatus*

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In this study, 66 genotypes of French bean were screened against bean rust under glasshouse and field conditions, of which, 11 genotypes with different degree of resistance/susceptibility were selected for measuring the slow rusting components. Both, incubation period and latent period were significantly and negatively correlated with infection frequency, disease severity and area under disease progress curve (AUDPC). Infection frequency was significantly and positively correlated with disease severity and AUDPC. The AUDPC was also significantly and positively correlated with disease severity while negatively correlated with uredium size. Factor analysis calculated using principal components revealed that factor I and II explained 65.97 and 15.45 per cent of the total variance, respectively. On the basis of scoring, it could be inferred that the five germplasm accessions *viz*., EC400406, EC755318, EC405210, EC400442 and EC400390 having low and negative factor I scores demonstrated slow rusting characteristics *i.e*., longer incubation and latent periods, lower infection frequency, smaller uredia and less disease severity against bean rust. These germplasm lines might have slow rusting genes and therefore, can be effectively used in breeding programs for developing rust resistant cultivars.

Key Words: AUDPC, Infection frequency, Latent period, *Phaseolus vulgaris*, Rust, Slow rusting resistance

Introduction

Rust (*Uromyces appendiculatus*) is a serious disease of French bean (*Phaseolus vulgaris* L.) throughout the world that causes significant yield reduction due to premature defoliation of the infected plants (Gupta *et al*., 2008; Liebenberg and Pretorius, 2010). The disease usually appears in Himachal Pradesh during the last week of July to the second week of August when crop is in the flowering or pod formation stage. This period usually coincides with slightly warmer and humid weather, which favours growth, reproduction and spread of rust pathogen. Losses in green pods ranging from 4.7 to 69 per cent have been reported due to this pathogen (Devi *et al*., 2019). Though this disease can be kept in check with the application of fungicides, but these have some limitations like environmental pollution, residual effect, high cost of application and health hazards. Bean rust can be effectively controlled by deploying resistant varieties, however the resistance does not remain effective for too long especially if it is conferred by major genes. Major gene resistance breakdown is a frequent phenomenon because plant pathogens such as *Uromyces appendiculatus* evolve into new and more virulent isolates through mutation or sexual recombination (Stavely 1984).

Slow rusting is an effective way to achieve durable rust resistance and appears to be race non-specific and more durable than major gene resistance (Ohm and Shaner, 1976). Slow rusting retards disease development in the field even when the infection type appears susceptible. Most of the work in slow rusting has been carried out in wheat where it has been found associated with a longer latent period, reduced pustule (uredium) size, and fewer uredia per unit area.

Therefore, this study was carried out with an objective to determine the mechanism of slow rusting resistance, to ascertain the role of different components of slow rusting in French bean and to investigate the

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relationship of these components to each other and to the rust severity.

Materials and Methods

Selection of genotypes for slow rusting

Sixty six French bean genotypes were evaluated under glasshouse as well as natural epiphytotic conditions for two consecutive crop seasons, *i.e.,* 2015 and 2016. Out of these, ten genotypes *viz.,* EC400390, EC400406, EC400408, EC400411, EC400435, EC400442, EC405210, EC405224, EC755318 and EC283179, each having a different degree of resistance/ susceptibility were selected based on disease reaction under field conditions along with highly susceptible cv. 'Falguni' as check.

Pathogen selection and inoculum multiplication

Uromyces appendiculatus isolate collected from Nauni, Solan (Himachal Pradesh, India) was used as test pathogen and mass multiplied on susceptible variety Falguni. The plants of Falguni were raised in pots (12.5 cm diameter) filled with sterilized mixture of fine loam and farmyard manure (3:1). After 15 days of sowing, plants were inoculated with urediniospores of *U. appendiculatus* by leaf coating method. The inoculated plants were sprayed with fine mist of water and incubated in saturated humidity chambers for 24 h, then shifted to the glasshouse at 20±2°C. Fifteen days post inoculation (dpi) urediniospores were collected and stored at -20°C for further studies.

Raising of genotypes for inoculation

To evaluate the components of slow rusting resistance, plants of different French bean genotypes were raised in separate plastic pots as mentioned above. In each pot, 6-8 seeds of each genotype were sown and after seedling emergence three plants per pot were maintained. For inoculation, urediniospores of *U. appendiculatus* were suspended in Tween 20 (0.05%, v/v) at a concentration of 2.0×10^4 urediniospores/ml. Inoculation was done by spraying urediniospores suspension with an atomizer on both sides of the leaves. After inoculation, fine mist of water was sprayed on these plants with pneumatic hand sprayer (Aspee Agro Equipment Pvt. Ltd.) to provide leaf wetness. The inoculated plants were then kept in humid chamber to provide high humidity and leaf wetness for 24 h and then shifted to glasshouse benches at ambient temperature (20±2°C) and observed regularly for the appearance of the symptoms.

Measurement of slow rusting components and analysis

Incubation and Latent period: Incubation period (number of days from inoculation to the first appearance of symptoms) was recorded as described by Van der Plank (1963). Number of days from inoculation to the appearance of 50% of the uredia, were recorded as latent period (Habtu and Zadoks, 1995).

Infection frequency: The number of uredia/cm^2 that appears on the leaf area after uniform inoculation is called infection frequency (Ohm and Shaner, 1976). The number of uredia /cm^2 were counted at the end of latent period by making 1 cm^2 slit on luggage tag. The one cm^2 slit was then placed on basal, middle and end of the leaves and no. of uredia in the slit were counted (Singh, 1985).

Uredium size: The size of 10 randomly selected uredia was measured with the help of stage and ocular micrometer. The ocular scale (a glass disc engraved in 0.01 mm scale and fitted in the eye piece lens) was focused after superimposing it on the stage micrometer scale under stereo-zoom microscope. The number of ocular micrometer divisions coinciding with the fixed number of stage micrometer scale was recorded. The value of each division in the eye piece was calculated as per the formula given by Birchen (1963). The ocular scale was calibrated at 4.5 × 15X magnification and all the measurements were made by taking length and width (mm). The area of the uredium (mm^2) was calculated as the product of π (Pi) ×½ length ×½ width as adopted by Kochman and Brown (1975).

Disease severity (%): Rust severity was estimated 14 times with the aid of modified 0-5 scale of Stavely (1983) as 0 = no disease, 1 = 0.1–5%, 2 = 5.1–10.0%, 3 = 10.1–25.0%, 4 = 25.1–40% and 5 = > 40% leaf area covered with rust uredia and per cent disease severity was calculated according to formula proposed by McKinney (1923).

$$\text{Disease severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Area under disease progress curve (AUDPC): The area under disease progress curve was calculated from disease severity as proposed by Shaner and Finney (1977).

$$AUDPC = {}^{n}\sum_{i=1} [(Y_{i+n1} + Y_i)/2] [X_{i+1} - X_i]$$

Where,

Yi = disease severity (per unit) at i^{th} observation,

Xi = time (days) at i^{th} observation, and

n = total number of observation

Apparent infection rate (r): The apparent infection rate (r) was calculated by the formula given by Van der Plank (1963).

$$r = \frac{2.3}{t_2 - t_1} \log \left\{ \frac{X_2}{1 - X_2} - \log \frac{X_1}{1 - X_1} \right\}$$

Where, r = apparent infection rate per unit per day, t_1 = first date of recording disease intensity, t_2 = second date of recording disease intensity, X_1 = disease intensity at time t_1, X_2 = disease intensity at time t_2, 2.3 = constant value

Data analysis. The data were analysed using the XLSTAT software to perform simple correlation, multiple regression and Factor analysis. Factor analysis using principal component utilizing correlation matrix was used to estimate the factor loadings. Eigenvalue >1 are considered for interpretation and indicate the proportion of total variance explained by each principal component (Sharma, 1996).

Results

Incubation period (IP) and Latent period (LP)

Incubation and latent periods varied significantly between in slow rusting and fast rusting French bean genotypes (Table 1). IP ranged from 168 h in Falguni to 196 h in EC400442. The genotypes *viz.,* EC400390, EC400406, EC755318 and EC405210 have shown longer incubation period of 175 to 185 h against fast rusting lines. LP ranged from 216 h (Falguni) to 288 h (EC400442) and the genotypes *viz.,* EC400390, EC400406, EC405210 and EC755318 showed longer latent period of 240, 252, 245 and 252 h, respectively.

Infection frequency

Of the eleven genotypes studied, number of uredia was minimum on the line EC755318 (4.0/cm^2) followed by EC400406 EC400442 and EC400390 with 6.33, 8.0 and 12.0 per cm^2, respectively, which differed significantly from other genotypes. The maximum infection frequency (63.67/cm^2) were recorded on Falguni (Table 1, Fig.1).

Uredium size

Size of uredia varied from 0.03 mm^2 in EC400442 to 1.53 mm^2 in EC755318. The genotypes *viz.,* EC400442, EC400411, EC400406, EC405224, EC400435 and EC400390 showed much smaller sized uredia ranging from 0.03 to 0.16 mm^2 (Table 1, Fig.1).

Area under disease progress curve (AUDPC) and apparent infection rate (r)

The data (Table 1) pertaining to area under disease progress curve and apparent infection rate (r) revealed that the values of both were minimum in the line EC400442 followed by EC400406, EC755318, EC405210 and EC400411 while the values were maximum in Falguni.

Disease severity (%)

Minimum disease severity was recorded in the genotype EC400442 (4.62%) followed by EC400406 and EC755318 each with 6.90 per cent, whereas, disease severity was maximum in Falguni (70.19%) followed by genotype EC400408 (68.82%) which were statistically at par with each other (Table 1).

Correlation and regression between components of slow rusting

The correlation coefficients between slow rusting components indicated that the incubation period (IP) was significantly and positively correlated with latent period (LP). Both IP and LP were significantly and negatively correlated with infection frequency, AUDPC and disease severity (Table 2). Infection frequency was significantly and positively correlated with AUDPC and disease severity. AUDPC was significantly and positively correlated with disease severity while negatively correlated with uredium size.

The multiple regression equations were also computed with disease severity as the dependent variable, and each of the components as independent variables.

$$Y = 313.31 - 1.81 X_1 + 0.13 X_2 + 0.63 X_3 - 5.64 X_4$$

Where, Y = Disease severity; X_1 = Incubation period; X_2 = Latent period; X_3 = Infection frequency; X_4 = Uredium size.

The coefficient of determination (R^2=0.905) between disease severity and group of independent variables indicated that 90.00 per cent bean rust severity was due to these variables, whereas rest of the variation was due to unexplained (error variation) factors and/or the factors not included in the investigation.

Factor analysis calculated using principal components

Table 1. Components of slow rusting resistance to *U. appendiculatus* in different genotypes of French bean

Genotypes	Incubation period (h)	Latent period (h)	Infection frequency (uredia/cm^2)	Uredium size (mm^2)	AUDPC	Apparent infection rate	Disease severity (%)
***Slow rusting*:**							
EC400406	184	252	6.33	0.08	176.50	0.069	6.90 (15.19)
EC755318	182	252	4.00	1.53	176.50	0.069	6.90 (15.20)
EC405210	185	245	16.33	0.27	434.88	0.089	22.42 (28.24)
EC400442	196	288	8.00	0.03	106.94	0.050	4.62 (12.34)
EC400390	175	240	12.00	0.16	817.74	0.101	38.86 (38.55)
***Fast rusting*:**							
EC400408	173	223	46.33	0.42	1477.71	0.130	68.82 (56.05)
EC400411	186	235	38.33	0.05	518.08	0.091	23.77 (29.15)
EC400435	175	225	42.67	0.12	1014.10	0.106	44.23 (41.67)
EC405224	175	225	48.67	0.10	1341.42	0.125	62.23 (52.06)
EC283179	185	235	22.00	0.29	624.92	0.098	29.48 (32.87)
Falguni	168	216	63.67	0.22	1655.36	0.134	70.19 (56.90)
LSD $_{0.05}$	(1.85)	(3.04)	(5.25)	(0.01)			(2.31)
±SE(m)	0.63	1.03	1.78	0.003			1.06

Figures in parentheses are arc sine transformed values

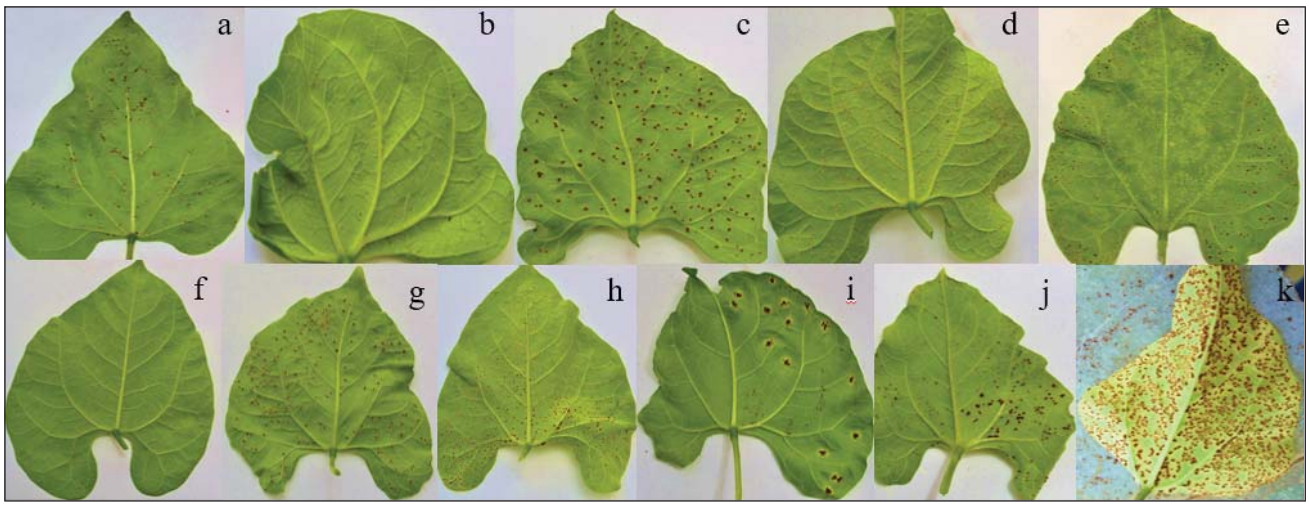

Fig. 1. leaves of genotypes, a) EC400390, b) EC400406, c) EC400408, d) EC400411, e) EC400435, f) EC400442, g) EC405210, h) EC405224, i) EC755318, j) EC283179 and k) Falguni, showing infection frequency

Table 2. Correlation coefficients among the components of slow rusting resistance in French bean genotypes

Variables	IP	LP	IF	US	AUDPC	r	DS
IP	1.00						
LP	0.86*	1.00					
IF	-0.69*	-0.80*	1.00				
US	-0.05	0.06	-0.30	1.00			
AUDPC	-0.87*	-0.83*	0.90*	-0.21	1.00		
r	-0.36	-0.09	0.19	-0.10	0.50	1.00	
DS	-0.85*	-0.83*	0.88*	-0.22	0.99*	0.52*	1.00

IP- Incubation period (h); LP- Latent period (h); IF- Infection frequency (No. of pustules/cm^2); US- Uredium size (mm^2); AUDPC- Area under disease progress curve; r-Apparent infection rate; DS- Disease severity (%)
*Significant at P≤ 0.05

revealed that factor I and II, each having eigenvalue greater than unity, explained 65.97 and 15.45 per cent of the total variance, respectively (Table 3). Factor I represented differences between average effects of infection frequency, AUDPC and disease severity which were interpreted as fast rusting characters. Incubation period and latent period which showed high and negative weights for factor I were interpreted as slow rusting characters to pathogen reproduction. Factor II represented differences for apparent infection rate and latent period which were interpreted as fast rusting characters. These two factors together accounted for 81.41 per cent of the total variance.

Table 3. Scores, eigen value and cumulative variance of the two major factors obtained from the PCA of slow rusting components

Variables	Factor I	Factor II
Incubation period	-0.900	0.068
Latent period	-0.879	0.362
Infection frequency	0.899	-0.206
Uredium size	-0.178	-0.076
Area under disease progress curve	0.995	0.087
Apparent infection rate	0.434	0.812
Disease Severity	0.991	0.114
Eigenvalue	4.617	1.081
Per cent variance	65.97	15.45
Cumulative (%)	65.97	81.41

Scoring of genotypes based on factor analysis

On the basis of scoring, it could be inferred that the germplasm lines *viz*., EC755318, EC400442, EC400406, EC400390 and EC405210 having negative values of Factor I scores possessed slow rusting characters while the genotypes *viz*. Falguni, EC400408, EC405224, EC400435, and EC283179 had positive and high factor I score values. The interpretation of factor II was not clear so it was not found very useful in differentiating between the genotypes (Table 4).

Thus only five French bean germplasm lines *viz*., EC755318, EC400442, EC400406, EC400390 and EC405210 demonstrated slow rusting characteristics *i.e.,* longer incubation and latent period, lower infection frequency, smaller uredia and less disease severity, therefore, these lines might have slow rusting genes.

Discussion

In the present study, slow rusting French bean germplasm lines showed longer incubation and latent periods than

Table 4. Scoring of genotypes based on factor analysis

Genotypes	Factor I	Factor II
Slow rusting:		
EC400406	-0.306	0.186
EC755318	-0.289	0.488
EC405210	-0.245	-0.593
EC400442	-0.238	-0.238
EC400390	-0.136	-0.178
Fast rusting:		
EC400408	1.115	-0.013
EC400411	0.066	-0.020
EC400435	0.320	0.264
EC405224	0.313	-0.011
EC283179	0.332	-0.213
Falguni	1.420	0.412

fast rusting lines. Similarly Habtu and Zadoks (1995) and Hegde (2001) reported extended latent period from 9.40 to 18.60 days and 9.17 to 16.22 days in bean and soybean genotypes, respectively and opined that the extended latent period played a major role in partial resistance.

Infection frequency, another important component of slow rusting, indicated that 11 genotypes differed in their ability to produce number of uredia/cm^2 leaf area. The germplasm lines with longer incubation and latent period exhibited uredial density of 4.00 to 16.33/cm^2 of leaf area, whereas the genotypes with shorter incubation and latent period had higher number of uredia (22.00 to 63.67/cm^2) which in turn reflected in high AUDPC and disease severity. Statler and McKey (1987) and Statler and Grafton (1988) recorded fewer number of uredia in resistant cultivars as compared to susceptible ones among French bean cultivars. Similarly lesser uredia/cm^2 in resistant bean cultivars against *U. appendiculatus* have been reported by Shaik (1985) and Habtu and Zadoks (1995), which is an important component of slow rusting mechanism.

Smaller sized uredia were noticed in slow rusting cultivars as compared to fast rusting cultivars except in germplasm line EC-755318 which was found to have largest sized uredia. Similar results were obtained with bean rust (Habtu and Zadoks, 1995) and leaf and stem rusts of wheat (Mabrouk *et al*., 2019).

In our studies, lowest AUDPC value (106.94) was recorded for the genotype EC-400442 and the maximum (1655.36) for Falguni. Differences in AUDPC have successfully been used to evaluate cultivars of wheat (Mabrouk *et al*., 2019) and soybean against rust (Hegde,

2001). AUDPC has been reported a more reliable criterion for quantifying slow rusting in wheat by Luthra *et al.* (1991). We observed that apparent infection rate was of little value in determining relative levels of slow rusting ability. Patil (1996) pointed out that "r" values were not as useful as AUDPC values, hence, 'r' should be coupled with AUDPC values to arrive at better conclusion in the mechanisms of slow rusting.

The genotypes *viz.*, EC400442, EC400406, EC755318, EC405210 and EC400390 showed lower disease severity, hence, these may be carrying slow rusting resistance. Van der Plank (1963) suggested the importance of disease severity in measuring the slow rusting mechanism in compound interest diseases like rusts. Rashid and Bernier (1991) recorded lower disease severity in the slow rusting cultivars of faba bean which had varied degree of tolerance.

Correlation between disease severity with the infection frequency, AUDPC and apparent infection rate was significant and positive whereas it was strong and negative with latent period. Similar strong correlations were also reported by Safavi *et al*. (2010) for wheat rust.

On the basis of factor analysis, two factors were extracted, where factor I represented the differences between average effects of disease severity, infection frequency, uredium size, infection rate and AUDPC. The factor I had high and negative weights for incubation period and latent period which were interpreted as slow rusting components. Scoring on the basis of this analysis revealed that germplasm lines namely, EC400442, EC400406, EC755318, EC405210 and EC400390 had low and negative scoring as compared to fast rusting genotypes. Similar scoring of genotypes on the basis of principal component analysis has been reported by Sandhu and Gupta (2001) in *Brassica* spp.

Conclusion

This study presents a preliminary report on the genotypic differences observed for the slow rusting components in 11 French bean genotypes. Based on the values of slow rusting components and their univariate analysis for different genotypes, it can be concluded that the French bean germplasm lines, *viz*., EC400442, EC400406, EC755318, EC405210 and EC400390 possessed slow rusting characters and can be effectively used in breeding programs for developing rust resistant cultivars. The genotypes exhibited variations in the level of slow rusting resistance which further suggests that there could be diversity in the number of genes involved and/or their effect in conferring the slow rust resistance against *Uromyces appendiculatus* in these genotypes.

Conflict of Interest: The authors declare that they have no conflict of interest.

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