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SCREENING OF CANOLA GERMPLASM AGAINST *ALBUGO CANDIDA* AND ITS EPIDEMIOLOGICAL STUDIES

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A B S T R A C T

White rust of crucifers is an important disease caused by *Albugo candida* (Pers. ex Lév.) Ktze. Twelve lines of *Brassica juncea* L. czern. et coss and *Brassica napus* L. var. *oleifera* were screened against white rust under field conditions. Correlation and regression studies were carried out between white rust severity and environmental factors (temperatures and relative humidity). There was a significant positive correlation of white rust severity with minimum, maximum temperature and relative humidity. *Brassica juncea* 44S01 and *Brassica napus* Sps-N7/26 were "highly resistant" lines. One degree increase in maximum and minimum temperature increased the maximum disease severity by 5.40, 5.88, 6.09, 6.87, 7.88% and 5.58, 6.04, 6.31, 7.06, 8.28% in *B.juncea* Sps-J10, *B. juncea* 44S45, *B. juncea* Pac-437, *B. juncea* Y065045 and *B. juncea* 7X1105-4 respectively and vice versa. Maximum disease severity 6.535, 7.119, 7.487, 8.385 and 9.390% in *B. juncea* Sps-J10, *B. juncea* 44S45, *B. juncea* Pac-437, *B. juncea* Y065045 and *B. juncea* 7X1105-4 respectively was observed by one percent increase in relative humidity and vice versa. The present study revealed that one degree or one percent increase or decrease in environmental parameters change the severity of disease.

Keywords: *Albugo candida*, *Brassicaceae, Brassicae juncea, B. napus,* Correlation.

INTRODUCTION

Canola commonly known as sweet mustard is genetically modified from rapeseed. It belongs to family Brassicae which consists of 330 genera and 3700 species worldwide but in Pakistan there exist 92 genera and 250 species (Jafri, 1973). Some of the well-known species of canola are *Brassica oleraceae*, *B. napus* L., *B. rapa* L., *Armoracia rusticana* P. Gaertn etc. Canola species *B. napus* L. and *B. juncea* L. both are conventional sources of oil and have great importance as food ingredients all over the world especially in South Asia (Mishra *et al.,* 2009). These are traditionally grown in all parts of Pakistan to meet domestic oil demands. Major producers of canola and rapeseed in international markets are China, Canada, European Union, India, Ukraine and Australia while very minute amount produced by Mexico, Pakistan and Europe. In Pakistan, canola rapeseed is the second major source of oil after cotton and fulfills about 17% of domestic demand of edible oil. In Pakistan, canola production remained 88 thousand tonnes over 172 thousand hectares and oil production was 76,000 tonnes. In Punjab province the target for canola was 95,680 tonnes with sowing area 58,700 ha (Anonymous, 2009). It contains less amount of two undesirable constituents: erucic acid (more than 2%) and traces of sulphur compound (glucosinolate more than 30 µmol/g) compared to rapeseed. In Canola oil there are two types of fatty acids: one has the highest level of mono and unsaturated fatty acids other having lowest level of saturated fatty acids which lower the cholesterol level (Przybylski and Mag, 2002).

Brassica L. crops are highly infested by various fungal pathogens and insects, while bacterial and viral diseases have little impact on their yield (Abdel-Farida *et al.,* 2009). Among these, fungal diseases such as White rust,

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downy mildew, powdery mildew, Alternaria blight, club rot of crucifer, black leg, Verticillium wilt, damping off etc. play significant role in decreasing the yield of canola. *Albugo candida* (Pers. ex Lév.) Ktze., the cause of white rust belongs to family *Albuginaceae* J. Schrot., which is considered a vulnerable disease attacking at least 29 genera resulting in great reduction in yield (Armstrong, 2007). The pathogen is an obligate parasite and survives by means of oospores in diseased plant debris and in the soil for more than 20 years (Gupta *et al.,* 2004). White rust infected canola crop may produce 37-47% less pods and 17-32% fewer seed, which affects the overall production (Bains and Jhotey, 1980). The fungus infects all the aerial parts of plants and caused two types of infection, general or systemic and local diseased plants become covered with white, chalky, blisters like pustules, frequently with hypertrophy and distortion of infected plant part. Systemic infection results in the formation of malformed meristem and inflorescence which give rise to racemes commonly described as staghead (Mishra *et al.,* 2009).

Due to increasing demand of low cholesterol oil good production/ high yield of canola is required. But now-a-days white rust is becoming a serious disease triggered by environmental factors resulting in less production of canola in Pakistan. Environment is an important parameter in the disease triangle to study after the presence of susceptible host and inoculum because the prevailing environmental conditions can speed up or reduce the infection and developmental process of any disease. In Pakistan little work has been done on epidemiological studies and screening of canola and rapeseed varieties/ lines. Keeping in view the importance of this disease, the objective of this study was to screen available canola and rapeseed germplasm and observed the impact of epidemiological factors on development of white rust disease.

MATERIALS AND METHODS

Preparation of land and sowing: The research work was conducted at research area of Department of Plant Pathology, Faculty of Agriculture Science and Technology, Bahauddin Zakariya University (Multan, Pakistan) during 2011-12. A total of 12 local canola lines were used in trial. Eight varieties/ lines of *B. juncea* i.e. 44S01, Y065045, Pac-437, Sps-J10, 44S45, 7X1105-4, 8PS-J79 and BRJ-7057, and four varieties/ lines of *B. napus* i.e Sps-N7/26, RBN-03255, Aup-4-10 and omega II were collected from Oilseed Research Station (ORS) Khanpur, Pakistan. The experiment was carried out in Randomized Complete Block Design (RCBD) in triplicate. Seedbed was prepared by ploughing two times followed by planking. The crop was sown at the rate of 0.81 kg/ha by hand drill method during 15th November, 2011. Two lines of each variety were sown in each plot (plot size 3.65 m × 4.57 m) with 0.45 m plant to plant distance and bed size of 0.76 m at the depth of 2-3cm. All the cultural practices, i.e. thinning, hoeing, weeding, manuring, fertilizers and irrigation were applied at their appropriate time for keeping plants in their good health. Four irrigations were applied, two thinnings were done, one after 1st and second after 2nd irrigation respectively.

Artificial inoculation of pathogen: Samples of canola showing symptoms of white rust were collected from different areas of Punjab i.e. Kahror pakka and Sadiqabad. Eighty (80) g of diseased plant parts showing typical symptoms of disease were ground and mixed with 1 L distilled water to prepare spore suspension. First the rubbing of plant leaves was done with boric acid and spores suspension was uniformly sprayed on 50-60 days old plants with the help of knap sack sprayer. Pustules of white rust were begun to appear on lower side of leaves after 7-8 days of inoculation.

Disease severity: Disease severity data on *Brassica* leaves was taken from ten plants which were selected and tagged randomly from each row. Symptoms of white rust were observed on 20th January 2012. The data regarding severity was recorded for six weeks on weekly basis after the initiation of disease symptoms in the field by using 0-5 disease rating scale suggested by Sangeetha and Siddaramaiah (2007) (Table 1). Percent disease index of white rust was evaluated by the formula given by Wheeler, (1969).

$$\text{Percent Disease Index (PDI) \%} = \frac{\text{Sum of individual rating}}{\text{No. of leaves x Maximum examined disease scale}} \times 100$$

Table 1. Scale for symptom of white rust of crucifers (Sangeetha and Siddaramaiah, 2007)

Scale	Description of the symptom
0	Leaves free from infection
1	Small white raised rust pustules covering <5% leaf area
2	Small white raised rust pustules covering 5.1-10% leaf area

3	Small white raised rust pustules join together covering 10.1-25% leaf area
4	Small white raised rust pustules join together to form irregular patches covering 25.1-50% leaf area
5	Small white raised rust pustules join together to form irregular large patches covering <50% leaf area

Environmental parameters: Data of environmental parameters of daily minimum and maximum temperature and relative humidity from 13th January 2012 to 24th February 2012 was collected from meteorological office situated at Multan airport.

Statistical analysis: The mean data of environmental parameters was subjected to correlation and regression analysis with white rust severity using Minitab 15 Statistical software.

RESULTS

Screening of canola germplasm against white rust of crucifer: Screening of resistant varieties/ lines for white rust requires growing of available canola germplasm in the field. Twelve lines were inoculated with spore suspension of *Albugo candida* through knapsack sprayer. The final data was recorded after 8 days of inoculation when initial symptoms of bending and twisting of the affected plants were observed. The data on white rust severity was started from 20th January 2012 up to 24th February 2012 when the disease was maximum with one week interval.

Out of 12 lines, *B. juncea* 44S01 and *B. napus* Sps-N7/26 were "highly resistant" lines with no disease symptoms throughout the study. Whereas *Brassica juncea* BRJ-7057 (10%) was moderately resistant. *Brassica juncea* Y065045 (94%) and *B. juncea* 7X1105-4 (98%) showed maximum disease severity and classified as "susceptible" lines. *Brassica juncea* 8PS-J79, *B. napus* RBN-03255, *B. napus* Aup-4-10, *B. napus* Omega- II, *B. juncea* 44S45, *B. juncea* Sps-J10 and *B. juncea* Pac-437 with 36, 38, 44, 56, 72, 70 and 82 % severity were categorized into moderately susceptible lines respectively during entire study period (Table 1).

Table 1. Disease severity percentage and reaction of canola germplasm against white rust disease caused by *Albugo candida*.

Varieties	% Disease Severity	Response of variety
B.juncea 44S01	0%	R
B.napus Sps-N7/26	0%	R
B.napus RBN-03255	38 %	MS
B.juncea Y065045	94 %	S
B.juncea Pac-437	82 %	MS
B.juncea Sps-J10	72 %	MS
B.napus Aup-4-10	44 %	MS
B.juncea 44S45	70 %	MS
B.juncea 7X1105-4	98 %	S
B.juncea 8PS-J79	36 %	MS
B.juncea BRJ-7057	10 %	MR
B.napus Omega II	56 %	MS

Relationship of environmental parameters with white rust severity: Two lines *B. juncea* 44S01 and *B. napus* Sps-N7/26 had no correlation with environmental variables because of 0% disease severity. The correlation of white rust severity with maximum temperature, minimum temperature and relative humidity was statistically significant (Table 2).

Table 2. Effect of environmental variables on white rust severity in ten canola lines.

Varieties/ lines	Maximum Temperature	Minimum Temperature	Relative Humidity
B. napus RBN-03255	- 91.52 + 3.562 x r= 0.91*	- 46.55 + 3.715 x r= 0.92*	- 224.7 + 4.312 x r= 0.94*
B. juncea Y065045	- 159.9 + 6.866 x r= 0.90*	- 71.48 + 7.059 x r= 0.89*	- 420.8 + 8.385 x r= 0.94*
B. juncea Pac-437	- 144.7 + 6.090 x r= 0.89*	- 67.10 + 6.308 x r= 0.89*	- 378.8 + 7.487 x r=0.94*
B. juncea Sps-J10	- 125.9 + 5.403 x r= 0.92*	- 55.86 + 5.526 x r= 0.91*	- 327.6 + 6.535 x r=0.96*
B. napus Aup-4-10	- 78.61 + 3.356 x r= 0.90*	- 36.21 + 3.498 x r= 0.91*	- 205.0 + 4.079 x r=0.94*
B. juncea 44S45	- 145.5 + 5.883 x r= 0.93*	- 69.47 + 6.035 x r= 0.92*	- 365.3 + 7.119 x r=0.96*
B. juncea 7X1105-4	- 181.9 + 7.879 x r= 0.93*	- 83.52 + 8.283 x r= 0.94*	- 468.2 + 9.390 x r=0.95*
B. juncea 8PS-J79	- 83.42 + 3.173 x r= 0.88*	- 39.86 + 3.104 x r= 0.83*	- 204.1 + 3.878 x r=0.92*
B. juncea BRJ-7057	- 15.11 + 0.642 x r= 0.81*	- 6.265 + 0.626 x r= 0.77	- 40.84 + 0.808 x r=0.88*
B. napus Omega II	- 95.47 + 4.177 x r= 0.94*	- 41.39 + 4.277 x r= 0.93*	- 249.3 + 5.014 x r=0.97*

* = Significant (P<0.05)

There was a statistically significant correlation between maximum temperature and white rust severity on all *Brassica* lines. One degree increase or decrease in maximum temperature increased or decreased the severity of white rust by 0.64, 3.17, 3.36, 3.56, 4.18, 5.40, 5.88, 6.09, 6.87 and 7.88% in *B. juncea* BRJ-7057, *B. juncea* 8PS-J79, *B. napus* Aup-4-10, *B. napus* RBN-03255, *B. napus* Omega II, *B.juncea* Sps-J10, *B. juncea* 44S45, *B.*

juncea Pac-437, *B. juncea* Y065045 and *B. juncea* 7X1105-4 respectively (Table 2).

Similarly statistically significant correlation was observed between white rust severity and minimum temperature in 9 *Brassica* lines while one line *B. juncea* BRJ-7057 showed non-significant correlation. One degree increase in minimum temperature increased white rust infection by 0.63, 3.10, 3.72, 3.49, 4.28, 5.53, 6.04, 6.31, 7.06 and 8.28% in *B.juncea* BRJ-7057, *B.juncea* 8PS-J79, *B.napus* RBN-03255, *B.napus* Aup-4-10, *B.napus* Omega II, *B.juncea* Sps-J10, *B.juncea* 44S45, *B.juncea* Pac-437, *B.juncea* Y065045 and *B.juncea* 7X1105-4 respectively and vice versa (Table 2).

The relationship between disease severity and relative humidity was also statistically significant. One percent increase in relative humidity increased the severity by 0.81, 4.31, 3.88, 4.08, 5.01, 6.54, 7.12, 7.49, 8.39 and 9.39% in *B. juncea* BRJ-7057, *B. napus* RBN-03255, *B. juncea* 8PS-J79, *B. napus* Aup-4-10, *B. napus* Omega II, *B. juncea* Sps-J10, *B. juncea* 44S45, *B. juncea* Pac-437, *B. juncea* Y065045 and *B. juncea* 7X1105-4 respectively (Table 2).

DISCUSSION

Pakistan is an agriculture based country but production of canola is lower due to susceptible germplasm against white rust disease which contributed significantly in disease epidemics. The attack of this disease reduced the growth of the crop convincingly and resulted in yield losses.

An indirect method of zoospore suspension inoculation and directly by exposure to natural inoculation of *Albugo candida* spores on *Brassica juncea* genotypes was adopted in order to identify the useful sources of resistance in *Brassica* crop (Li *et al.,* 2008). The seeds of turnip rape were inoculated with oospores of *A. candida* before sowing (Verma and Petrie, 1980). This pretreatment of fungal spores actually reduced the white rust infection in field experiment, whereas inoculation of zoospores before inflorescence formation resulted in 10 fold increase in disease severity. Their results are in agreement with the outcome of this disease. The development of white rust on brassicaceous crops is highly effected by temperature, relative humidity and rainfall (Srikantaswamy *et al.,* 2006). The effect of temperature and disease severity on oospores germination of *Albugo candida* was checked and reported that foliar infection is important for the formation of zoospores to initiate secondary infection (Williams and Pound, 1963). Relationship of white rust severity and environmental factors were studied to predict losses in result of attack on leaves and pods (Chattopadhyay *et al.,* 2005). Temperature at a range 16-24°C with 40-97 % relative humidity favours the development of white rust disease on leaves while staghead formation takes place at 29°C (Chattopadhyay *et al.,* 2011). As incidence of white rust is increased with fluctuation in weather conditions, suitable temperature range of 15-30°C with average relative humidity of less than 65% along with occasional occurrence of rains causes the conducive development of disease (Raj *et al.,* 2008; Sangeetha and Siddaramaiah, 2007). The findings of this research are cognizant with their results. Ontogeny of *Albugo* and severity of white rust affected by environmental conditions, optimum temperature for development of disease varied between 12 to 18°C although disease severity decreased for temperature outside the given range (Correll *et al.,* 1994; Sullivan *et al.,* 2002). It was confirmed in the study that when temperature was 11.5 – 12.5°C, and average relative humidity was 75% along with frequent rains results in faster progression of rust pustules (Saharan *et al.,*1988). Relative humidity increases the release of *A. candida* spores, maximum spore release in the afternoon and minimum in the morning hours (Humpherson and Maude, 1982). Their results are in conformity with the findings of preset study. *A. candida* cause significant yield losses in *B. juncea*, *B. rapa* and some susceptible lines of *B. napus* in India, China and Australia (Mukherjee *et al.,* 2001). Few *B. juncea* lines were resistant to white rust attack (Meena *et al.,* 2011). These results are in favour of the findings of present research.

CONCLUSIONS

From a total of 12 lines of *B. juncea* and *B. napus* screened against white rust of crucifers, *B. juncea* 44S01 and *Brassica napus* Sps-N7/26 were highly resistant and *B. juncea* BRJ-7057 was moderately resistant line, whereas all other lines were susceptible. Sowing of crop at proper time and temperature will also help to escape disease. There is a need to use varieties/ lines that must be resistant to this disease in order to avoid any epidemic in future.

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