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Research Article

Genetics of resistance to stem rust against Indian races in wheat varieties, Kundan and UP 2338

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Abstract
The parents, F_1 and F_2 individuals of the crosses Kundan x Agra Local, UP 2338 x Agra Local and Kundan x UP 2338 were screened to study the genetics of resistance in the Ug99-resistant varieties of wheat, Kundan and UP 2338 against two Indian stem rust pathotypes 40A (62G29) and 122 (7G11). Against race 40A (62G29), the variety Kundan did not have any resistance alleles, whereas, UP 2338 exhibited the presence of dominant resistance gene. The 195 F_2 individuals of the cross UP 2338 × Agra Local and Kundan × UP 2338 segregated at a ratio of 3(R):1(S). Against stem rust pathotype 122 (7G11), the variety Kundan displayed monogenic dominant resistance. The resistance x resistance cross Kundan x UP 2338 also displayed resistance reaction in the F_2 generation with no segregation indicating the presence of common gene in both the parents (Kundan and UP 2338) conferring resistance against the pathotype 122.

Key words
Triticum aestivum, *Puccinia graminis* Pers. f. sp. *tritici*, genetics, resistance, Ug99 resistant varieties

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple food crop grown all over the world in an area of about 225 million hectare (Singh *et al.,* 2008a and b). India is the second largest producer of wheat in the world. Wheat is the second most important cereal food crop after rice in India and serves as staple food crop for millions of people. In India wheat is grown on about 29.25 million hectare with production and productivity of about 85.93 million tons and 2.93 t/ha respectively (Anonymous, 2011).

There are many biotic and abiotic factors which affect the wheat crop, among the biotic factors, the rust pathogen assume particular significance as these can change genetically and multiply rapidly causing epidemic situation and thereby resulting into yield losses (Ahamed *et al.,* 2004). The wheat rusts (leaf rust, yellow rust and stem rust) have historically been the diseases of great importance and they have significantly influenced the development of human civilization (Roelfs *et al*., 1992 and McIntosh *et al*., 1995).

Stem rust also known as black rust of wheat caused by fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., historically is known to cause severe devastation periodically and was most feared disease in various countries in all continents where wheat is grown (Singh *et al.,* 2008). However, this rust was controlled for last several decades through host plant resistance by identifying number of rust resistance genes from wheat and related species and introgressing some of them into wheat varieties.

Since 1999 it has been observed that the stem rust is gaining importance due to appearance of new race, commonly known as Ug99 in wheat growing areas of Kenya and Ethiopia causing susceptibility to popular wheat varieties (Singh *et al.,* 2008). Eighty percent of all Asian and African wheat varieties are known to be susceptible to Ug99 (Singh *et al.,* 2008). Due to a narrow genetic base and continuously evolving pathogen races, resistant varieties become susceptible to the disease when grown over vast areas (Assefa and Fehrmann, 2004). Host plant resistance is the most economical method of controlling it because resistance reduces or eliminates the need for fungicide application (Singh, 2004). The most effective approach to control this disease is to develop varieties carrying more than one resistance gene, thereby providing prolonged resistance against several races (Pederson and Leath, 1988). To date, a number of stem rust (*Sr*) resistance genes have been identified and assigned to specific chromosomes (McIntosh *et al.,* 2003). Thus, introducing new resistance will allow the development of elite cultivars with broadened resistance to stem rust. Therefore, an experiment was conducted during 2008-09 and 2009-10 with the main objective to study the genetics of the seedling resistance to stem rust races 40A (62G29) and 122 (7G11) in Ug99 resistant wheat varieties Kundan and UP 2338.

Material and Methods

In this investigation two Ug99 resistant varieties, Kundan (Tonari71 x NP890) and UP 2338 (UP368/VL421//UP262), and a susceptible variety Agra Local were used. Two crosses were made by crossing Kundan and UP 2338 as a female parents with a susceptible variety Agra Local used as a male parent and the third cross was made by crossing Kundan (resistant) as female and UP 2338 (resistant) as male during 2008-09 in the field. In the following season F_2 populations were produced by selfing the F_1 generations at National Phytotron Facility, Indian Agricultural Research Institute (IARI), New Delhi.

In India one of the most commonly prevalent virulent pathotype of *Puccinia graminis.* f. sp. *tritici*, 40A (62G29), and the weak pathotype 122 (7G11), were used for screening of parents, F_1 and F_2 individuals at seedling stage, under glass house conditions at Division of Genetics, IARI, New Delhi. The initial inoculums of these two pathotypes were obtained from Directorate of Wheat Research (DWR) Regional Station, Flowerdale, Shimla, India and the inoculums were multiplied on susceptible cultivar Agra Local in isolation under glass house conditions.

For seedlings testing with stem rust pathotypes, ten days old parents, F_1 and F_2 seedlings grown in pan trays were inoculated with 2 races, 40A (62G29) and 122 (7G11) to two different portions of the same leaf (space inoculation) (Prabhu and Luthra, 1992) with talcum mixed urediospores of individual pathotype. In the case of Kundan × Agra Local, only single race inoculation using race 122 was made since both parents were susceptible to race 40A. Inoculated seedlings were kept in humidity chambers for 36 hours and then shifted to glass house benches at 23 $\pm$ 2°C temperature. Rust reaction was recorded 15 days after inoculation following the scale proposed by Stakman *et al.*, (1962). Chi-square (χ^2) test was employed to test goodness of fit of observed and expected frequency in segregating generations.

Results and Discussion

The crosses, resistant x susceptible type (UP 2338 x Agra Local) and resistant x resistant type (Kundan x UP 2338) F_2 populations including their parents and F_1 plants were screened for stem rust resistance at seedling stage against the race 40A. All the three crosses, two resistant x susceptible types (Kundan x Agra Local & UP 2338 x Agra Local) and one resistant x resistant type (Kundan x UP 2338), parents (Kundan, UP 2338 and Agra Local) as well as, F_1 and F_2 individuals were tested at seedling stage against stem rust pathotype 122.

Parents, UP 2338 showed resistant reaction, Kundan and Agra Local showed susceptible reaction with infection type '1', '3^+' and '33^+' respectively, to 40A pathotype of stem rust. Whereas, for 122 pathotype Kundan and UP 2338, both displayed resistant reaction with infection type ';' (fleck) and the susceptible parent, Agra Local produced susceptible reaction with infection type '33^+'. The observations are presented in the Table 1.

Against the 40A pathotype, F_1 plants of the cross UP 2338 x Agra Local produced resistant reaction indicating the dominant nature of the resistant gene. The segregation pattern in F_2 populations fits in 3 (resistant) : 1 (susceptible) ratio (χ^2 = 1.06), out of 195 F_2 plants, 140 were resistant and 55 were susceptible, indicating presence of single dominant gene in UP 2338 against the stem rust pathotype 40A (Table 2). The cross Kundan x UP 2338 also confirmed the presence of a single dominant gene in UP 2338 for resistance to 40A. The F_2 population segregated in 142R : 53S in a 3R : 1S ratio (Table 2). Previously Bahadur *et al*., (2004) reported the presence of 1 dominant resistance gene in UP 2338 against pathotype 40A (62G29) and was confirmed by the present study. Sharma and Singh (2000) studied inheritance of resistance to stem rust in the crosses involving Selection 212 and the susceptible wheat Agra Local and Chinese Spring at the seedling stage with pathotypes 122 and 40A. Reported, single recessive gene controlled resistance to both pathotypes in Selection 212. Ghazvini *et al*., (2012) elucidated, the inheritance of resistance to Ug99 in Norin 40 is controlled by single gene. Steffenson *et al*., (2009) reported single gene in Q21861 confers resistance to race TTKSK.

Similarly for stem rust race 122, the F_1 seedlings of the crosses Kundan x Agra Local, UP 2338 x Agra Local were resistant indicating dominant nature of the resistant gene. Out of 194 F_2 plants of the cross Kundan x Agra Local tested, 145 plants produced resistant reaction and 49 plants produced susceptible reaction, which fits well with 3 (resistant) : 1 (susceptible) ratio with χ^2 value of 0.006 confirming the presence of single dominant gene in Kundan against pathotype 122. But all the 194 F_2 individuals of the cross UP 2338 x Agra Local did not segregate into susceptible type, they were all resistant (Table 2). This non-Mendelian inheritance may be because F_2 population was derived from more than one F_1 plants orinsufficient population or it may also because of some other unknown reasons. Even though UP 2338 was used as a female parent, the failure of segregation in F_2

could not be ascribed to the possible selfing of parent UP 2338 for absence of hybridity with Agra Local. This is because, the same cross (same set of individuals) segregated against race 40A (Table 2).

Mago *et al*., (2005) while validating the PCR markers for *Sr24* and *Sr26* found that, Sr24#12 marker was completely linked to *Sr24*, the Sr24#50 marker failed to predict the presence of *Sr24*. Although the Sr24#50 marker amplified from the resistant parent, the same band amplified from only one of the 36 resistant doubled haploid lines and from none of the susceptible segregants and they explained this non-Mendelian inheritance could be because of the mapping family, being derived from more than one F_1 plant and that a deletion or a rare recombination event in one F_1 generated a shorter Agropyron segment (lacking the Sr24#50 marker) that was inherited by most of the progeny.

The F_1 plants of the cross Kundan x UP 2338 (resistant x resistant) displayed resistance reaction against stem rust race 122. But F_2 individuals did not segregate for susceptibility as all seedlings produced resistance reaction as a consequence of the presence of same stem rust resistance gene in both the parents, Kundan and UP 2338 conferring resistance (Table 2). Karimi *et al*., (2010) while studying the inheritance of *Fusarium* wilt resistance in pigeonpea observed the resistant reaction in F_1 plants and lack of susceptible segregates among the F_2 populations from crosses between resistant × resistant parents suggests that the wilt resistant genes in the two genotypes ICEAP 00554 and ICEAP 00557 were allelic. Because F_1 populations were resistant to *Fusarium* wilt this was evidence that resistance is controlled by dominant. Bahadur *et al*., (2004) while analysing the genetics of resistance to stem rust in five bread wheat cultivars against three pathotypes of *Puccinia graminis* f. sp. *tritici viz.*, 21 (9G5), 40A (62G29) and 117-5 (166G22) found that, the F_2 seedlings of all the intercrosses of parents did not segregate for susceptibility to pathotype 21 (9G5). They reported, it is because of presence of common resistance genes in the cultivars.

Knott and McIntosh (1978) reported that resistance to several North American and Australian races of stem rust in Webster wheat is controlled by the same nearly recessive gene, which is designated *St30*. Hiebert *et al.*, (2011) studied the genetics of seedling resistance to Ug99 stem rust race in Canadian wheat cultivars Peace and AC Cadillac by producing two populations, an $F_{2:3}$ populations from LMPG/AC Cadillac and a doubled haploid (DH) population from RL6071/Peace and found that both populations were segregated for a single stem rust resistance (Sr) gene at the seedling stage. Prabhu and Luthra (1992) discovered 1 dominant and 1 recessive genes operating for resistance to each of the races 14A, 20A, 31 and 38A in wheat tester Heines VII, in contrary to the expected presence of 1 dominant resistance gene *Yr2*.

Sharma and Singh (2000) reported avirulence/virulence formulae for 40A and 122 pathotypes are as follows. Pathotype 40A avirulence to *Sr13*, *Sr21*, *Sr24*, *Sr30*, *Sr37* and virulence to *Sr5*, *Sr7b*, *Sr8a*, *Sr9b*, *Sr9e*, *Sr11*, *Sr18*, *Sr19*, *Sr20*, *Sr28*, pathotype 122 avirulence to *Sr8a*, *Sr9e*, *Sr24*, *Sr28*, *Sr30*, *Sr37* and virulence to *Sr5*, *Sr7b*, *Sr9b*, *Sr11*, *Sr13*, *Sr18*, *Sr19*, *Sr20*, *Sr21*. Both these pathotypes are avirulent on *Sr25*, *Sr26*, *Sr27*, *Sr31*, *Sr32* and virulent on *Sr6*, *Sr9a*, *Sr9d*, *Sr9f* and *Sr16*. Based on the published data (Sharma and Singh 2000 and Das *et al*. 2006) confirm the presence of *Sr31* in UP 2338. Pretorius *et al*. (2000) reported virulence of Ug99 for *Sr31* gene. This study here confirms that in addition to *Sr31*, some other novel resistance gene(s) may be present in UP 2338 and Kundan, which needs to be verified. Further analysis of stem rust resistance in both Kundan and UP 2338 could provide wheat breeders with additional genetic resources for developing cultivars that are more broadly resistant to stem rust, including Ug99. It is however cautioned here that whenever such transfers are carried out, the transfer of resistance against Ug99 also needs to be confirmed. Till molecular markers are developed for Ug99 resistance, phenotyping of selected resistant lines to Indian races needs to be carried out at Kenyan facility against Ug99.

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Table 1. Reactions of parental varieties against 2 stem rust races 40A (62G29) and 122 (7G11) of wheat

Race	Name of variety	Variety reaction	Infection type
40A	Kundan	S	3^+
	UP2338	R	1
	Agra Local	S	33^+
122	Kundan	R	;
	UP2338	R	;
	Agra Local	S	33^+

*R, Resistant; S, Susceptible

Table 2. Reactions of F_1 and F_2 seedlings against 2 stem rust races 40A (62G29) and 122 (7G11) of wheat

Race	Cross	F_1 reaction	No. of F_2 seedlings Observed		Expected F_2 ratio (R:S)	No. of F_2 seedlings Expected		χ^2 value	P value
			R	S		R	S		
40A	UP 2338 X Agra Local	R	140	55	3:1	146.25	48.75	1.06	0.303
	Kundan X UP 2338	R	142	53	3:1	146.25	48.75	0.49	0.483
122	Kundan X Agra Local	R	145	49	3:1	145.5	48.5	0.006	0.938
	UP 2338 X Agra Local	R	194	0					
	Kundan X UP 2338	R	191	0					

*R, Resistant; S, Susceptible