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Genetics of Adult Plant Resistance to Leaf Rust in Egyptian Wheat

A.I. Fahmi*, M. Nazim, S.Z. Khalifa** and W.M. El-Orabey*****

* *Genetics Dept., Fac. of Agric., Minufiya Univ., Shebin El-Kom, Egypt.*

** *Agric. Botany Dept., Fac. of Agric., Minufiya Univ., Shebin El-Kom, Egypt.*

*** *Wheat Dis. Dept., Plant Pathol. Inst., Agric. Res. Centre.*

This investigation was carried out to identify leaf rust resistance genes in five Giza wheat cultivars, *i.e.* Giza 155, Giza 157, Giza 164, Giza 165 and Giza 167. Plants of F2 segregations from the crosses between these varieties and Near-Isogenic Thatcher lines, *i.e. Lr* 9, *Lr* 12, *Lr* 13, *Lr* 24, *Lr* 34, *Lr* 35 and *Lr* 36 were tested under field conditions with a mixture of physiologic races. Giza 155 and Giza 167 had the adult plant resistance gene *Lr* 9, Giza 165 and Giza 167 had *Lr* 13, and Giza 165 had *Lr* 24. While cv. Giza 157, Giza 164 and Giza 167 have *Lr* 34. The cv. Giza 157 and Giza 164 had *Lr* 35 and *Lr* 36.

Key words: Leaf rust, monogenic lines, plant resistance and wheat.

The resistance of wheat cultivars to leaf rust disease caused by *Puccinia triticina* Eriks is the most economic and environmentally preferable method to manage this disease. Development and management of durable resistant cultivars has not been entirely successful. New or previously undetected races of the leaf rust fungus frequently have overcome the resistance of new resistant cultivars. The resistance used is based on genes which are effective throughout the plant growth cycle. To date, 46 leaf rust resistance (*Lr*) genes have been identified in cultivated wheats and their wild relatives (McIntosh *et al.*, 1995), several of which are race specific in nature. Durable leaf rust resistance is worldwide and conditioned by adult plant resistance gene (s) (Roelfs, 1988). The inheritance of adult plant durable resistance has often been considered complex, but there is also an evidence that it is oligogenic (Barcellos *et al.*, 2000). Little is known about the adult plant leaf rust resistance genes present in Egyptian wheats.

The identification of gene(s) conferring leaf rust adult plant resistance would be a significant step towards a better control of this disease (Liu and Kolmer, 1997; Kolmer, 1996; Hussain *et al.*, 1999 and Saini *et al.*, 1999). Therefore, the main objectives of this investigation were to identify the genes governing resistance to leaf rust in some Egyptian wheat Giza cultivars and to determine the number of genes controlling resistance to leaf rust in each variety under study.

Materials and Methods

Five Egyptian wheat cultivars and seven Near-Isogenic Thatcher lines (NILs) for wheat leaf rust were tested for their reaction to leaf rust. The wheat cultivars

belonging to Giza cultivars were cv. Giza 155, Giza157, Giza 164, Giza 165 and Giza 167. While, the selected Thatcher NILs were *Lr* 9, *Lr* 12, *Lr* 13, *Lr* 24, *Lr* 34, *Lr* 35 and *Lr* 36. The seeds of the Thatcher NILs were provided by the Cereal Disease Laboratory, St. Paul, Minnesota, USA.

The parental cultivars and the Thatcher NILs were grown in the farm of the Faculty of Agriculture, Minufiya University, Shebin El-Kom. They were grown on three successive dates at 15 days intervals to overcome differences in the time of flowering. The Thatcher NILs were used as male parents for crosses with each of Giza cultivars to obtain the F1 seeds. Any doubtful F1 plants were discarded and the others were harvested separately. The F1 seeds were grown in the following season in rows 4 m long and 30 cm apart and spaced 30 cm in order to facilitate production of F2 seeds. Parents, F1 and F2 plants were also grown in plots, each plot of the parents and F1 contained six rows 3.5 m long and 20 cm between rows. Each plot of the F2 contained eight rows 4 m long spaced 30 cm and seeds were 25 cm apart, each row therefore containing 15 plants. All plots were surrounded by a spreader area sown with a mixture of two wheat cultivars highly susceptible to leaf rust, Giza 139 and Thatcher.

Inoculation and disease assessment:

For field inoculation, the spreader plants were mist sprayed with water and dusted with a 20:1 talcum powder: spores mixture of the most prevalent leaf rust physiologic races in the area, races 77 and 57. Dusting was carried out in the early evening (at sunset) before dew formation and when air was not yet still. The inoculation of all plants was carried out at booting stage according to the method of Tervet and Cassell (1951). Data of leaf rust severity were recorded at the adult stage of the test plants using the scale of Peterson *et al.* (1948).

Leaf rust severity (%) was recorded for each cultivar from the time of rust first appearance then every seven days until the early dough stage (Large, 1954) using the modified Cobb's scale (Peterson *et al.*, 1948). Plant reaction (infection type) expressed in five types (Stakman *et al.*, 1962). The infection types were; immune= (O), resistant= (R), moderately resistant= (MR), moderately susceptible= (MS) and susceptible= (S).

Leaf rust severity (%) was recorded for each F_2 plant at the appearance of the first leaf rust pustule (Large, 1954). Plants of the F_2 for each cross were grouped into six categories depending on their percentage of disease severity under field conditions: 1) very resistant (VR) with no rust visible infection; 2) resistant (R) with low infection up to 20% rust severity; 3) moderately resistant (MR) with 21% up to 40% rust severity; 4) moderately susceptible (MS) with 41% up to 60% rust severity; 5) susceptible (S) with 61% up to 80% rust severity; and 6) very susceptible (VS) with 81% to 100% rust severity. The first three categories were considered resistant phenotypes and the latter three groups (41% to 100%) are considered as susceptible phenotypes.

For identification of the adult plant leaf rust resistance genes in each cross, goodness of fit of the observed to the expected ratio of the phenotypic classes was tested using Chi-square (χ^2) analysis according to Steel and Torrie (1960).

Results

The detailed results of crosses between Giza cultivars and the Thatcher NILs are shown in Table 1 and summarised in Table 2. All of the 82 F2 plants of the cross between the adult plant resistant *Lr* 9 and Giza 155 and the 84 between *Lr* 9 and Giza 167 were very resistant (VR) under field conditions, indicating that Giza 155 and Giza 167 have the leaf rust resistance gene *Lr* 9.

Table 1. Segregation and chi square analysis (3:1 ratio) of F_2 plants from the crosses between the monogenic lines and wheat cultivars under field conditions

Cross	Infection type						No. of * F_2 plants		χ^2	*P* value**	Significance
	VR	R	MR	MS	S	VS	Res	Sus			
Giza 155 X *Lr* 9	29	51	2	0	0	0	82	0	-	-	N.S.
Giza 157 X *Lr* 9	17	29	19	25	0	0	65	25	0.370	0.542	
Giza 165 X *Lr* 9	3	39	0	7	14	0	42	21	2.333	0.1266	
Giza 167 X *Lr* 9	30	48	6	0	0	0	84	0	-	-	N.S.
Giza 155 X *Lr* 12	0	0	7	11	27	20	18	47	-	-	-
Giza 157 X *Lr* 12	0	0	10	20	18	22	10	60	-	-	-
Giza 164 X *Lr* 12	0	14	3	17	13	15	17	45	-	-	-
Giza 165 X *Lr* 12	0	0	6	14	15	25	6	54	-	-	-
Giza 155 X *Lr* 13	0	8	0	37	18	0	8	55	-	-	-
Giza 157 X *Lr* 13	2	3	21	31	9	0	26	40	-	-	-
Giza 164 X *Lr* 13	0	0	9	24	18	15	9	57	-	-	-
Giza 165 X *Lr* 13	7	53	10	0	0	0	70	0	-	-	N.S.
Giza 167 X *Lr* 13	15	60	5	0	0	0	80	0	-	-	N.S.
Giza 155 X *Lr* 24	11	43	0	11	0	0	55	11	2.444	0.118	3:1
Giza 157 X *Lr* 24	0	11	8	27	24	0	19	51	-	-	-
Giza 164 X *Lr* 24	0	0	4	65	0	0	4	65	-	-	-
Giza 165 X *Lr* 24	29	55	0	0	0	0	84	0	-	-	N.S.
Giza 157 X *Lr* 34	33	42	0	0	0	0	75	0	-	-	N.S.
Giza 164 X *Lr* 34	19	51	0	0	0	0	70	0	-	-	N.S.
Giza 165 X *Lr* 34	0	20	12	14	16	0	32	30	0.064	0.799	3:1
Giza 167 X *Lr* 34	29	48	0	0	0	0	77	0	-	-	N.S.
Giza 157 X *Lr* 35	10	57	5	0	0	0	72	0	-	-	N.S.
Giza 164 X *Lr* 35	13	72	0	0	0	0	85	0	-	-	N.S.
Giza 165 X *Lr* 35	0	32	10	13	0	0	42	31	11.876	0.00065	3:1
Giza 167 X *Lr* 35	0	45	15	23	0	0	60	23	0.325	0.568	3:1
Giza 157 X *Lr* 36	24	46	0	0	0	0	70	0	-	-	N.S.
Giza 164 X *Lr* 36	33	47	0	0	0	0	80	0	-	-	N.S.

* Res= Resistant, Sus= Susceptible.

** *P* values higher than 0.05 indicate no significant of χ^2.

Table 2. Summary of genes present in Giza cultivars understudy

Gene / Cultivar	*Lr* 9	*Lr* 12	*Lr* 13	*Lr* 24	*Lr* 34	*Lr* 35	*Lr* 36
Giza 155	+	-	-	-	nt	nt	nt
Giza 157	-	-	-	-	+	+	+
Giza 164	nt	-	-	-	+	+	+
Giza 165	-	-	+	+	-	-	nt
Giza 167	+	nt	+	nt	+	-	nt

(+) = The gene is present.
(-) = The gene is absent.
(nt) = The gene not tested.

F2 plants of crosses between *Lr* 9 and the rest of the tested cultivars, *i.e.* Giza 157 and Giza 165, segregated to 65R:25S and 42R:21S respectively. These segregations fit a 3R:1S ratio for Giza 157 but not for Giza 165, but it can be concluded that neither of these cultivars expresses *Lr* 9.

The F2 plants of crosses between *Lr* 12 and the four wheat cultivars Giza 155, Giza 157, Giza 164 and Giza 165 segregated at the ratios 18R:47S, 10R:60S, 17R:45S and 6R:54S respectively, none of which fit a 3R:1S ratio, indicating that they do not express *Lr* 12.

All of 70 and 80 F2 plants of the crosses between *Lr* 13 and the cvs. Giza 165 and Giza 167 respectively showed resistance indicating that they express *Lr* 13. The F2 plants of the crosses between *Lr* 13 and the rest of the cultivars, Giza 155, Giza 157 and Giza164 segregated at 8R:55S, 26R:40S and 9R:57S, respectively, again not fitting a 3R:1S ratio but indicating that they do not have the leaf rust resistance gene *Lr* 13.

All of 84 F2 plants from the cross between Giza 165 and *Lr* 24 were resistant, indicating expression of *Lr* 24. F2 plants of the crosses between *Lr* 24 and the cvs. Giza 155, Giza 157 and Giza 164 segregated at 55R:11S, 19R:51S and 4R:65S respectively, again not fitting a 3R:1S ratio indicating that they did not express *Lr* 24 resistance.

All of 75, 70 and 77 F2 plants of the crosses between *Lr* 34 and the cvs. Giza 157, Giza 164 and Giza 167 were resistant and therefore expressed *Lr* 34 resistance but cv. Giza 165 segregated at 32R:30S not fitting a 3R:1S ratio and not therefore expressing this gene either.

The F2 plants for the crosses between *Lr* 35 and the cvs. Giza 157 and Giza 164 were all resistant and therefore express *Lr* 35 whilst Giza 165 did not and therefore do not express this gene. The cross between *Lr* 35 and cv. Giza 167 segregated at a ratio of 60R:23S which was a good fit to the ratio of 3R:1S, but still indicating that this cultivar did not express *Lr* 35.

All of F2 from cvs. Giza 157 and Giza 164 crossed to *Lr* 36 were resistant and therefore they expressed *Lr* 36.

Discussion

The adult plant resistance genes are the most important in terms of widespread distribution and durability. Therefore, the focus has been on the resistance genes expressed at the adult stage, theoretically reducing the selection pressure for pathogen virulence.

The five Giza cultivars were crossed to all seven Thatcher NILs, but some of the crosses were not successful. Plants from the other F2 populations from all successful crosses showed a range of leaf rust severity. For analysis, the plants with up to 40% disease severity were grouped into the resistance category and 41-100% disease severity were grouped into the susceptible category.

***Lr* 9:**

This gene was isolated from *Triticum umbellulatum* (Soliman *et al.*, 1963) and it was mapped to chromosome 6BL. The results of this study showed the expression of this gene in the two cultivars Giza 155 and Giza 167 and no expression in cvs. Giza 157 and Giza 165. This gene was found in the old Egyptian cultivars and it has remained in the genetic materials since the 1970's. It is an important gene adapted to the Egyptian environment and therefore desirable in Egyptian breeding programmes.

***Lr* 12:**

This gene was originally isolated from the cv. Exchange (Dyck *et al.*, 1966). The resistance gene *Lr* 12 conferring adult-plant resistance was located on chromosome 4B (Dyck *et al.*, 1966). It was also found in the cv. Chinese Spring and occurs in several Eastern United States winter wheat varieties (Dyck and Samborski, 1979). F2 data revealed that this gene is neither expressed in the Egyptian genotypes or in the Egyptian environment.

***Lr* 13:**

This gene was originally isolated from the cv. Frontana (Dyck *et al.*, 1966). It is located on chromosome 2BS. It was found in many cultivars such as Manotou, Neepawa, Chris and Sinton (Dyck and Samborski, 1979). In the present study, F2 results indicated that this gene was found in Giza 165 and Giza 167. However, Giza 155, Giza 157 and Giza 164 cultivars did not express it, nor did they show a 3:1 ratio which may be due to the partial suppression of this gene in different backgrounds. The gene is clearly effective under the Egyptian environment but it is only expressed in recently developed cultivars and is not found in older Egyptian genotypes.

***Lr* 24:**

This gene was shown to be effective at seedling and adult stages. It was derived from *Agropyron elongatum*, located on chromosome 3DL and linked to *Sr*24 gene (Browder, 1973). Kolmer (1993), Long *et al.* (1993) and Martens and Dyck (1988) concluded that, in several European countries, United States and Canada. The *Lr* 24 gene is not a durable source of resistance and should be used only in combinations with other, *Lr* genes. However, in India it is known to be very effective against all the prevalent leaf rust virulence at the adult stage (Sawhney, 1985a, Kochumadhavan *et al.*, 1988). In this study it was found only in Giza 165.

***Lr* 34:**

Lr 34 is the most important, widespread distributed and durable. Roelfs (1988) indicated that the genes *Lr* 12 and or *Lr* 13 in combination with *Lr* 34 conferred durable resistance to leaf rust. This gene has been found in a number of wheat cultivars collected from diverse locations. Kolmer *et al.* (1991) mentioned that adult plant resistance genes *Lr* 13 and *Lr* 34 in many hard red spring wheats bred for leaf rust resistance in North America. In addition, Dyck (1991) attributed the adult plant resistance of the cv. Chinese Spring and Sturdy to the interaction of *Lr* 12 and *Lr* 34. Dyck (1994) identified *Lr* 34 from wheats from Iran, China, Afghanistan, Lebanon, Russia, Argentina, Tunisia and France. Shang *et al.* (1986) found *Lr* 34 in wheats from Manchuria and India. It is also remarkable that *Lr* 34 has continued to condition an effective level of resistance despite being in cultivars that have been extensively grown for extended periods of time in many wheat growing areas throughout the world (Kolmer, 1996).

Dyck (1987) located *Lr* 34 in chromosome 7D of wheat. Singh (1992) indicated that the *Lr* 34 is linked to a stripe rust resistance gene *Yr* 18. It is also tightly linked with barley yellow dwarf virus (BYDV) (Singh, 1993). In the present study, four cultivars; Giza 157, Giza 164, Giza 165 and Giza 167 were tested for the presence of the gene *Lr* 34. F2 results indicated that no segregation for three cutivars which indicates the presence of the *Lr* 34 in Giza 157, Giza 164 and Giza 167. This result is in accordance with the findings of Kolmer (1996) who reported that this gene is widespread conferring an effective level of resistance for extended periods of time. Giza 157 was released in 1970's, so *Lr* 34 is a relatively old gene in our cultivars and it is still effective in recent cultivars such as Giza 167 which was released in 1995. The cultivar Giza 165 showed a ratio of 1:1 instead of 3:1. This deviation from the ratio 3:1 may be due to the phenomenon of partial suppression. This gene is clearly important and should be considered in all breeding materials. Further studies are needed to understand the function of this gene.

***Lr* 35:**

Lr 35 was originally derived from *Triticum speltoides* (Kerber and Dyck, 1990). Resistance expressed by *Lr* 35 becomes noticeable at the second-leaf stage and is fully expressed after the sixth-leaf stage. F2 results demonstrated its expression in Giza 157 and Giza 164, while it was not expressed in Giza 165 and Giza 167. *Lr* 35 and another gene, *Sr* 39, are located on chromosome 2B with a recombination value of 3.0 % between them (Kerber and Dyck, 1990). Giza 167 showed 3:1 ratio while Giza 165 did not show fit with this ratio, which probably again indicated a partial suppression of this gene in this specific background. This suppressive effect was observed by Nelson *et al.* (1997) who suggested the presence of a *Triticum tauschii* gene on chromosome arm 2DS that suppresses resistance conferred by the *Lr* 23 gene on 2BS.

***Lr* 36:**

The *Lr* 36 gene was originally derived from *Triticum speltoides* (Dvorak and Knott, 1990). It was mapped to chromosome 6BS. In this study, two cultivars were tested and it was found in both. Thus this gene is effective in the Egyptian

environment and causes resistance at the adult stage. The gene was also effective at the seedling stage.

In conclusion, the adult plant resistance in the Egyptian cultivars under study are controlled by a group of genes including *Lr* 9, *Lr* 12, *Lr* 13, *Lr* 24, *Lr* 34, *Lr* 35 and *Lr* 36. Some of these genes are in Egyptian germplasm for a long time as they were found in the new and old cultivars, for example *Lr* 9 in Giza 155 and Giza 167 and *Lr* 34 was present in Giza 157 and Giza 167. With the exception of *Lr* 12, these genes are often found in combinations.

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وراثة المقاومة في طور البلوغ لصدأ الأوراق في القمح المصري

عبد المجيد ابراهيم فهمى* ، محمد نظيم سيد أحمد** ، السعيد زكى خليفه** ، وليد محمد العرابى***

* قسم الوراثة - كلية الزراعة – جامعة المنوفية.

** قسم النبات - كلية الزراعة – جامعة المنوفية.

*** قسم أمراض القمح – مركز البحوث الزراعية.

أجريت هذه الدراسة بهدف تعريف جينات المقاومة امرض صدأ الأوراق فى طور البلوغ فى خمسة أصناف قمح وهى جيزة ١٥٥ ، جيزة ١٥٧ ، جيزة ١٦٤ ، جيزة ١٦٥ ، جيزة ١٦٧. كان اهم النتائج المتحصل عليها بفحص انعزالات نباتات الجيل الثانى الناتجة من التهجين بين الأصناف الخمسة تحت الدراسة و مبعة سلالات حاملة لجينات فردية تحت ظروف الحقل أن الصنف جيزة ١٥٥ يحتوى على الجين *Lr* 9 و الصنف جيزة ١٥٧ و الصنف جيزة ١٦٤ يحتوى كل منهما على نفس الثلاث جينات وهى *Lr* 36 ,*Lr* 35 ,*Lr* 34 بينما الصنف جيزة ١٦٧ يحتوى على الجينات *Lr* 34 ,*Lr* 13 ,*Lr* 9.