Physiological and Molecular Plant Pathology 104 (2018) 48–57

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

Biochemical, histopathological and genetic analysis associated with leaf rust infection in wheat plants (*Triticum aestivum* L.)

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ARTICLE INFO

Keywords:
Wheat
Leaf rust
Lr genes
Enzymes activity
Anatomical structure

ABSTRACT

The response of Egyptian wheat cultivars (Gemmeiza-7, Gemmeiza-10, Gemmeiza-11 and Gemmeiza-12) against leaf rust was studied at adult stage at two locations during two seasons. The results differentiated cultivars to susceptible and resistant depend upon the epidemiological parameters; FRS (%), AUDPC and r-value. The resistant cultivars, (Gemmeiza-10 and Gemmeiza-12) have the resistant genes; *Lr10* and *Lr19*. However, Gemmeiza-7 and Gemmeiza-11 haven't any resistant genes. The discoloration of superoxide and hydrogen peroxide was decreased in moderately resistant and resistant cultivars compared to the susceptible cultivars. Also, electrolyte leakage increased in susceptible cultivars. Catalase and peroxidase activities were increased in resistant cultivars. Our results were confirmed with the anatomical studies which proved that epidermis thickness, mesophyll and phloem tissues were decreased in susceptible cultivars. Therefore, this explains why these cultivars still resistant till now.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most essential crops worldwide, especially in Egypt [2]. There are many biotic stresses affects the yield of wheat crop, such as yellow, leaf and stem rusts [2,3]. Leaf rust (*Puccinia triticina* f.sp. *tritici)* is considered the widespread rust disease. It became a serious disease, that causes a severe loss in grain yield of wheat plants [22]. The importance of such disease depends, mainly, on the evolution and appearance of virulent races of the pathogen in its populations and their ability to breakdown or overcome the resistance gene(s) of the host. Thus, the commercial wheat cultivars have suffered from severe leaf rust epidemics during the last few decades from the perspective of change in climate conditions in relation to the genetic makeup of both pathogen and host (Draz et al., 2015). This disease was the cause of eliminating several wheat varieties and discarding from agriculture in Egypt, *i.e.* Giza-158, Mexipak-69, Super-X and Chenab-70, because of their high susceptibility under field conditions in Egypt. In some cases, wheat varieties having monogenic type of resistance rapidly loses it's genetic resistance within a few years. Additionally, the cultivation of susceptible wheat cultivar in a large area, creating a reservoir for mutation and selection [25]. Therefore, the high successful and environmentally safe method of wheat protection against such disease is the host genetic resistance or growing resistant cultivars. Pyramiding genes is considered to be an effective control method, leading to durability of this genetic resistance against pathogens with low genetic diversity [23,24]. The pyramiding of the resistance genes is emphasized by the molecular markers techniques that have been applied for most resistance genes to leaf rust [7]. Gene postulation is the most common method to postulate the resistance genes in different cultivars at seedling stage [23]. Under any biotic and abiotic stresses conditions, microorganisms are correlated with production of ROS which prompts lipid peroxidation [15]. H_2O_2 and O_2 accumulation in the resistant cultivars resulted in less damage in the plant cells. Hence, H_2O_2 is a key role in resistance (Abdalaal et al., 2014). The antioxidant components are enzymatic and non-enzymatic. Enzymatic antioxidants include catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX) and polyphenoloxidase (PPO), as well as non-enzymatic antioxidants are glutathione, carotenoids and α-tocopherols. The up–regulation of CAT, POX and PPO plays vital role during elevated the ROS levels, thereby and protected plants from pathogen attack [18]. Either biotic or abiotic stresses led to changes in the membrane permeability (electrolyte leakage) (EL) of wheat plants [2]. In continuation of our earlier study, the main objective of this investigation was to find out the relationship between the susceptible and resistant cultivars inoculated

Abbreviations: Reactive oxygen species, ROS; superoxide, $O_2^{\cdot-}$; hydrogen peroxide, H_2O_2; catalase, CAT; peroxidase, POX; polyphenoloxidase, PPO; electrolyte leakage, EL

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https://doi.org/10.1016/j.pmpp.2018.09.004
Received 20 August 2018; Received in revised form 16 September 2018; Accepted 17 September 2018
Available online 19 September 2018

Table 1
Pedigree and Origin of grain resource of the four Egyptian wheat cultivars and the twenty Lr genes used in this study.

No.	Wheat cultivar	Pedigree
1	**Gemmeiza-7**	CMH74A .630/5X//SERI82/3/AGENT. GM4611-2GM-3GM-1GM-0GM.
2	**Gemmeiza-10**	MAYA74″S"/ON/1160–147/3/BB/G11/4/CHAT ″S"/5/CROW ″S″GCM 5820- 3 GM- 1 GM- 2 GM- 0 GM.
3	**Gemmeiza-11**	BOW″S"/KVZ″S"//7C/SERI82/3/GIZA168/SKHA61. GM7892-2GM-1GM-2GM-1GM-0 GM.
4	**Gemmeiza-12**	OTUS/3/SARA/THB//VEECMSS97Y00227S−5Y-010M-010Y-010M-2Y-1M-0Y-0 GM

Lr gene

No.	Lr gene	Origin of grain resource	No.	Lr gene	Origin of grain resource
1	***Lr1***	TC*6/Centenatrio (RL6003)	**11**	***Lr21***	TC*6/RL5406 (RL6043)
2	***Lr9***	Transefer/8*TC (RL6010)	**12**	***Lr28***	CS2D-2M
3	***Lr10***	TC*6/Exchange (RL6004)	**13**	***Lr29***	TC*6/CSAG#11 (RL6080)
4	***Lr11***	Kussar (W976)	**14**	***Lr30***	TC*6/Terenz10(RL6049)
5	***Lr12***	Exchange/6*TC (RL6011)	**15**	***Lr32***	TCLR32(RL4597)
6	***Lr13***	Manituou	**16**	***Lr33***	TC*6/P158548 (RL6057)
7	***Lr15***	TC*6/Kenya 1483 (RL6052)	**17**	***Lr34***	TC*6/P158548(RL6058)
8	***Lr16***	TC*6/Exchange (RL6005)	**18**	***Lr36***	E84018
9	***Lr19***	TC*7/Tr(RL6040)	**19**	***Lr45***	Secale cereale
10	***Lr20***	Thew (W203)	**20**	***Lr46***	Pavon 76

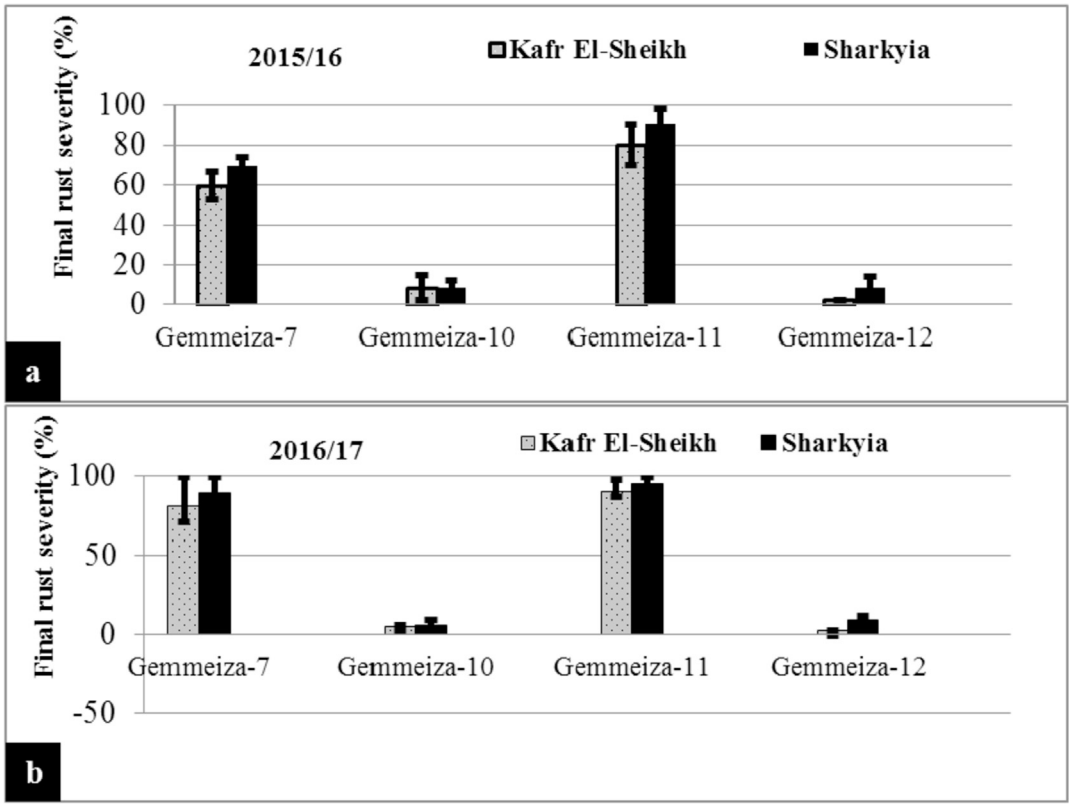

Fig. 1. Final rust severity (FRS %) of leaf rust infection on four Egyptian wheat cultivars, grown in Kafr El-Sheikh and Sharkyia locations, during 2015/16 and 2016/17 growing seasons.

with *Puccinia triricina* through biochemical changes, genetic analysis and anatomical structure.

2. Materials and methods

The present investigation was carried out at the experimental farms of Sakha Agric. Res. Station (Kafr El-Sheikh governorate) and Kafr El-Hamam Agric. Res. Station (Sharkyia governorate) in Wheat Diseases Research Department, Plant Pathology Research Institute (PPRI), ARC, Egypt, during 2015/2016 and 2016/2017 growing seasons. The Laboratory studies were carried out at Excellence Center and Plant Pathology and Biotechnology Lab. (certified according to ISO 17025, ISO 9001, ISO 14001 and OHSAS 18001) Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Egypt.

2.1. Field experiments

Four Egyptian wheat cultivars and twenty monogenic lines (Lr genes) (Table 1) were evaluated at the previous two locations, during 2015/16 and 2016/17 growing seasons. The wheat cultivars were sown in a randomized complete block design (RCBD) with 3 replicats. The experimental unit consisted of 3 rows (3 m long and 30 cm apart) with 5g grains rate for each row. The experiment was surrounded by 1.5 m belt, served as a spreader of leaf rust susceptible entries, *i.e.* “Morocco