Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 3 (3): 325-329, 2004
ISSN 1682-3974

Investigation for Resistance Traits in three Hexaploid Amphiploids (*Ttitipyrum*, *Triticales* and Wheats) to Seed Gall Nematode and Covered Smut Diseases

G.H. Shahidi Bonjar, [1]H. Shahsevand Hassani, N. Pakgohar and B. Barkhordar
Department of Plant Pathology, College of Agriculture, Bahonar University of Kerman, Iran
[1]Department of Agronomy and Plant Breeding, College of Agriculture, Bahonar University of Kerman, Iran

Abstract: Seed galls caused by *Anguina tritici* [Syn. *Anguillulina tritici* (Steinb.)] and covered smut caused by *Tilletia foetida* (Wallr.) are two important wheat pathogens worldwide. Presence of resistance to these pathogens were investigated in three Hexaploid Amphiploids of *Triticale* (AABBRR, 2n= 6x= 42), *Tritipyrum* (AABBE^{b}E^{b}, 2n= 6x= 42) and bread wheat (AABBDD, 2n= 6x= 42). Macaroni wheat (AABB, 2n= 4x= 28) and a double haploid wheat (AABBDD, 2n= 6x= 42) were included as controls. To gall nematode, line 4115 of *Triticale*, *Tritipyrum* lines of (Maconum/*Thinopyrum bessarabicum* x Creso/*Thinopyrum bessarabicum*, F_4), (Karim/*Th. bessarabicum* x Creso/*Th. bessarabicum* , F_3, F_5), double haploid variety (Omid, F_3), Macaroni wheat (Yavaros) and bread wheat varieties (Falat, Atila and Omid) were resistant and, *Triticale* (lines of 4115 and M_{45}), *Tritipyrum* (Stewart/*Th. bessarabicum* x Creso/*Th. bessarabicum*,F_4) and bread wheat (Roshan) were susceptible. To covered smut, line 4116 of *Triticale*, three lines of *Tritipyrum* (Stewart/ *Th. bessarabicum* x Creso/*Th. bessarabicum*,F_4), (Karim/*Th. bessarabicum* x Creso/*Th. bessarabicum*, F_3), (Macoum/*Th. bessarabicum* x Creso/*Th. bessarabicum*, F_4) and double haploid (Omid, F_3) were resistant and bread wheat cultivars (Falat, Atila and Roshan), Macaroni wheat (Yavaros), *Triticales* (lines of 4115 and M_{45}) and one *Tritipyrum* (Karim/*Th. bessarabicum* x Creso/*Th. bessarabicum*, F_4), were susceptible. It is conclusive that there is more genetic diversity in new lines of *Triticale* and *Tritipyrum* in comparison with that of Macaroni and bread wheat lines. Resistant lines found for these two diseases in this study, could be proper sources for producing genetically resistant and transgenic lines of present commercial wheat varieties. This the first report of resistance of the mentioned lines to *A. tritici* and *T. caries* in the first ever trial in Iran.

Key words: Hexaploid, amphiploid, *Triticale, Tritipyrum*

INTRODUCTION

Development of transgenic plants resistant to phytopathogens and biological control of plant diseases has received worldwide attention in recent years mainly as a response to public concern about the use of hazardous chemicals in the environment.

In modern agriculture, pesticide application is still an invaluable and effective method to control plant diseases. However, since use of agrochemicals is falling into disfavor because of environmental pollution and detrimental effects on a variety of nontarget organisms, potential use of microbes based biocontrol agents as replacement or supplements for agrochemicals has been addressed in many recent reports[1]. With the increased concern about conserving natural resources as air, soil and water, natural or biological control of plant diseases and development of resistant varieties; have received increased emphasis. Biological control of plant diseases is slow, gives few quick profits, but can be long lasting, inexpensive and harmless to life. Biocontrol systems do not eliminate neither pathogen nor disease but bring them into natural balance[2-4], while developing resistant varieties results more effective control.

Seed gall nematode (SGN) caused by *Anguina tritici* [Syn. *Anguillulina tritici* (Steinb.)] and covered smut (CS) caused by *Tilletia foetida* (Wallr.) are two important diseases of wheat worldwide[5-7]. They have been reported from most of wheat growing regions of Iran[8,9]. Pre-planting seed treatment is the classical control management for CS, however, several resistant varieties have been reported by Laughlin[6] and Czeeh[10]. Okhovat[11] reported that 21.71% of wheat plantation in Esfahan province of Iran had 4.73% infection with SGN. To reduce the use of chemical controls, breeding of plants for disease resistance and biological controls are the main

Corresponding Author: I.G.H. Shahidi Bonjar, Associate Professor of Plant Pathology, Department of Plant Pathology, College of Agriculture, Bahonar University of Kerman, Iran Tel: 0098 0913 342 0125 Fax: 0098-341-3222043 E-amil: shahidi@mail.uk.ac.ir

prospective worldwide. Tracking for resistant genes in new varieties is the avenue in developing for resistance via classic or genetic engineering methods[12-16]. Resistant varieties of corn, tomato and some other plants have been developed commercially[6]. In developing disease resistance in wheat, extensive research is in progress in Europe and USA[17,18].

At this study presence of resistant traits to CS and SGN were investigated in three Hexaploid Amphiploids of *Triticale* (AABBRR, 2n= 6x= 42), *Tritipyrum* (AABBE^{b}E^b, 2n=6x=42) and bread wheat (AABBDD, 2n= 6x= 42). Macaroni wheat (AABB, 2n= 4x= 28) and a double haploid wheat (AABBDD, 2n= 6x= 42) were included as controls.

MATERIALS AND METHODS

Plant sources: Seeds of the following wheats were prepared: a) Hexaploid varieties including Roshan, Omid, Atila and Falat; Tetraploid variety of Yavaros and a doubled haploid line of Omid (F3) from the Department of Seed and Seedlings Development, Agricultural Research Center, Kerman, Iran. b) *Triticale* lines of 4115, 4116 and M_{45} from Saline Research Center, Maragheh, Iran. c). Three new Amphiploid lines of *Tritipyrum* (Maconum/*Thinopyrum* bessarabicumx Creso/ *Thinopyrum bessarabicum*, F_4), (Karim/ *Th. bessarabicum* x Creso/ *Th. bessarabicum* , F_3, F_4 and F_5) from Gene Bank of John Innes Centre, Research Committee of Biology and Biotechnology of England. d) Infected seeds to CS and SGN of Falat variety from Department of Plant Protection, Research Center of Djehaad Keshavarzi, Ministry of Agriculture, Kerman, Iran.

Procedures for artificial infection: All healthy seeds were stratified for four weeks at 6°C in refrigerator, infested with the appropriate pathogen and sowed accordingly. Plastic containers (18x35 cm) containing sterilized sandy-clay soil and plant compost (3:1, w/w) were used for seed beds. For artificial infestation, 50 stratified healthy seeds were mixed with equal number of water-soaked and crushed infected seeds of each pathogen and sowed at 2 cm soil depths in the appropriate seed beds accordingly.

Evaluation for establishment of infection: Development of symptoms was evaluated in plant tops from early appearance throughout seed maturation by macroscopic and microscopic examinations. In the case of SGN, plants having leaf and spike curling and bearing seeds containing stages of *A. tritici* (eggs, larvae and adult stages) were evaluated as susceptible and plants lacking such symptoms considered as resistant. In the case of CS, plants having seeds filled with teliospores of *T. foetida* were evaluated as susceptible and plants lacking such symptoms considered as resistant. Microscopic examination of healthy seeds revealed normal amyloplasts in their endosperm (stained dark blue with 0.5% Leugol).

RESULTS AND DISCUSSION

Based on macroscopic and microscopic evaluations, susceptibility and resistance of tested wheat lines and varieties to NSG and CS are tested in Table 1.Heads and seeds of tested plants are shown in Fig. 1. Figure 2 shows comparison of infected seeds with gall nematode and healthy ones (A and B). In microscopic examinations, broken water soaked-seeds of infected varieties showed swarms of nematodes (Fig. 2C). Eggs and first larval stage (Fig. 2D) at early stage of seed ripening (Fig. 2D) and mature nematodes in ripe seeds of susceptible infected varieties were noticeable (Fig. 2E). Microscopic examinations of seeds of susceptible varieties infected with *Tilletia caries* showed teliospors in seeds (Fig. 3A) while seeds of resistant varieties showed normal amyloplasts (Fig. 3B).

Table 1: Results of Investigation for Resistance Traits in three Hexaploid Amphiploids (Ttitipyrum, Triticales and Wheats) to *Anguina tritici* and *Tilletia caries*

Lines and Varieties	*Anguina tritici*	*Tilletia caries*
Doubled haploid wheat (Omid, F3)	R	R
Triticum æstivum var. Falat	R	S
Triticum æstivum var. Atila	R	S
Triticum æstivum var. Omid	R	R
Triticum æstivum var. Roshan	S	S
Triticum durum var. Yavaros	R	S
Triticale 4116	S	R
Triticale M_{45}	S	S
Triticale 4115	R	S
Tritipyrum (St/b×Cr/b, F_4)*	S	R
***Tritipyrum (Ma/b×Cr/b, F_4)*RR**	R	R
***Tritipyrum (Ka/b×Cr/b, F3)*RR**	R	R
Tritipyrum (Ka/b×Cr/b, F5)	R	S

R: Resistant; S: Susceptible; (St/b×Cr/b, F_4)*: Stewart/ *Thinopyrum bessarabicum* x Creso/*Thinopyrum bessarabicum*, F_4; (Ma/b×Cr/b, F_4)*: Maconum/ *Thinopyrum bessarabicum* x Creso/ *Thinopyrum bessarabicum*, F_4; (Ka/b×Cr/b, F_5)*: Karim/ *Thinopyrum bessarabicum* x Creso/ *Thinopyrum bessarabicum*, F_3;? (Ka/b×Cr/b, F_5)*: Karim/ *Thinopyrum bessarabicum* x Creso/ *Thinopyrum bessarabicum* , F_5.

To gall nematode, line 4115 of *Triticale*, *Tritipyrum* lines of (Maconum/*Thinopyrum bessarabicum* x Creso/ *Thinopyrum bessarabicum*, F_4), (Karim/ *Th. bessarabicum* x Creso/ *Th. bessarabicum* , F_3, F_5), double haploid variety (Omid, F_3), Macaroni wheat (Yavaros) and bread wheat varieties (Falat, Atila and Omid) were resistant and, *Triticale* (lines of 4115 and M_{45}), *Tritipyrum* (Stewart/ *Th. bessarabicum* x Creso/ *Th. bessarabicum*,F_4) and bread wheat (Roshan) were susceptible. To covered smut, line

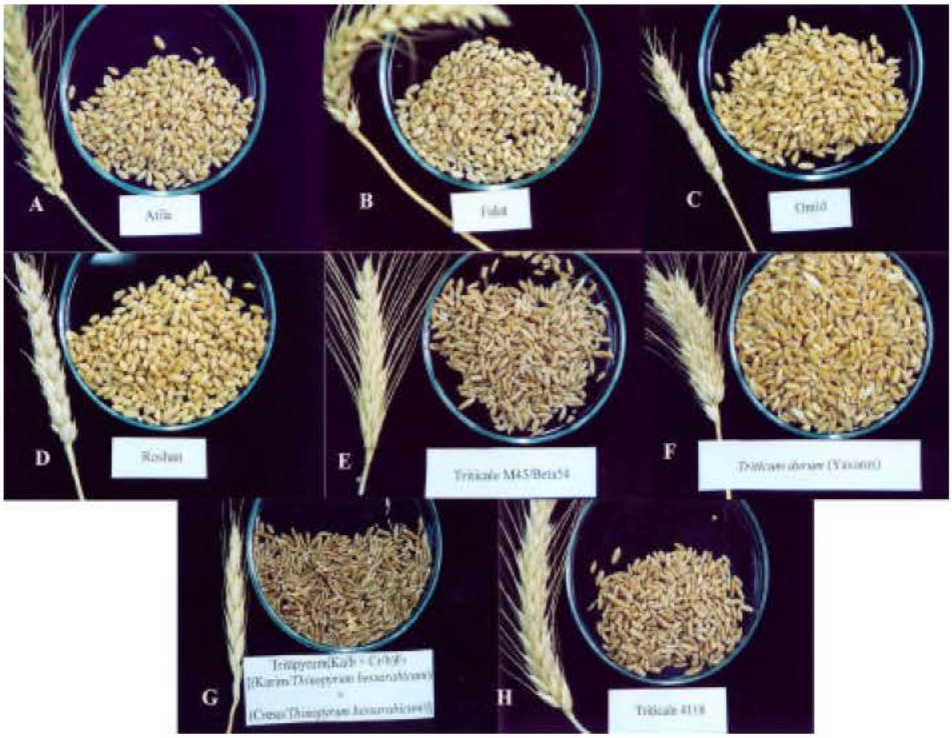

Fig. 1: Heads and seeds of tested plants. A) *Triticum aestivum* var. Atila, B) *Triticum aestivum* var. Falat, C) *Triticum aestivum* var. Omid, D) *Triticum aestivum* var. Roshan, E) *Triticale* 4116, F) *Triticum durum* var. Yavaros, G) *Tritipyrum* and H) *Triticale* 4115

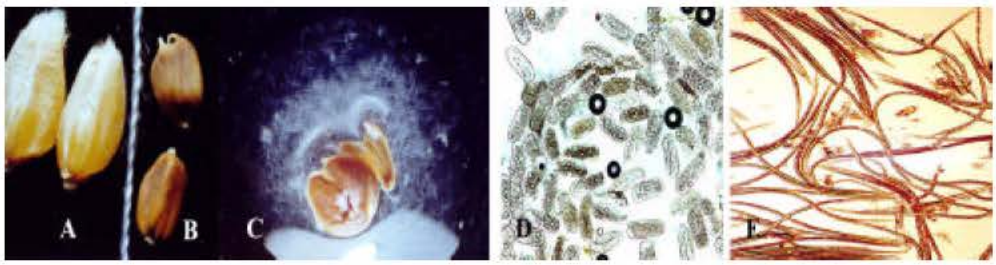

Fig. 2: Comparison of infected seeds with gall nematode (A) and healthy ones (B, 10 X). Broken water soaked-seeds (C, 40 X) showing swarms of nematodes. Eggs and first larval stage (D, 100 X) at early stage of seed ripening (D, 100 X) and mature nematodes in ripe seeds (E, 40 X)

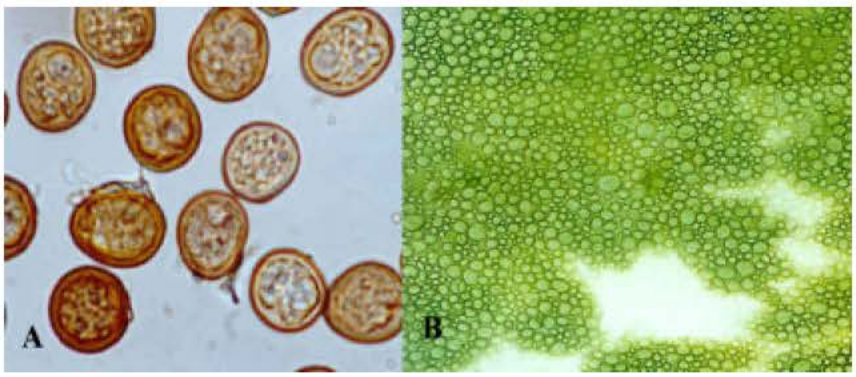

Fig. 3: Microscopic examinations of susceptible infected seeds showing teliospors of *Tilletia caries* (A, 400 X) and healthy resistant seeds showing normal amyloplasts (B, 100 X)

4116 of *Triticale*, three lines of *Tritipyrum* (Stewart/ *Th. bessarabicum* x Creso/ *Th. bessarabicum*,F_4), (Karim/*Th. bessarabicum* x Creso/*Th. bessarabicum*, F_3), (Macoum/*Th. bessarabicum* x Creso/*Th. bessarabicum*, F_4) and double haploid (Omid, F_3) were resistant and bread wheat cultivars (Falat, Atila and Roshan), Macaroni wheat (Yavaros), *Triticales* (lines of 4115 and M_{45}) and one *Tritipyrum* (Karim/*Th. bessarabicum*x Creso/ *Th. bessarabicum*, F_4), were susceptible. It is conclusive that there is more genetic diversity in new lines of *Triticale* and *Tritipyrum* in comparison with that of Macaroni and bread wheat lines. Resistant lines found for these two diseases in this study, could be proper sources for producing genetically resistant and transgenic lines of present commercial wheat varieties.

Expression of cloned genes in transgenic plants has provided evidence in plant defense[19]. The genes encoding many antifungal proteins are currently being used by agribusiness to create genetically modified plants that have increased fungal resistance in field[3]. Expression of cloned chitinase genes in transgenic plants has provided evidence of their role in plant defense[4]. Results of these findings may form the avenue for production of resistant transgenic-plants with recombinant DNA having resistant genes cloned from the resistant lines. This is the first report of resistance of the mentioned lines to *A. tritici* and *T. caries* in the first ever trial in Iran.

ACKNOWLEDGMENT

We acknowledge the cooperation of: Head of Department of Plant Protection, Research Center of Djehaad Keshavarzi, Ministry of Agriculture, Kerman, Iran. Mr. Amiri from the Department of Seed and Seedlings Development, Agricultural Research Center, Kerman, Iran. Mr. Marefat from Saline Research Center, Maragheh, Iran. Mr. Reader and Mr. Miller from Gene Bank of John Innes Centre, Research Committee of Biology and Biotechnology of England. Research Affairs Office of Bahonar Univ. of Kerman. The results of this research is dedicated to Mr. A. Afzalipour the founder of University at Kerman, God may bless him.

REFERENCES

1. Shimizu, M., Y. Nakagawa, Y. Sato, T. Furumai, Y. Igaroshi, H. Onaka, R. Yoshida and H. Kunoh, 2000. Studies on endophytic Actinomycetes (I) *Streptomyces* sp. isolated from Rododendron and its antifungal activity. J. General Plant Pathol., 66: 360-366.
2. Dhingra, O.D. and J.B. Sinclair, 1995. Basic plant pathology methods. CRC Press: USA, pp: 287- 296, 390- 391.
3. Selitrennikoff, C.P., 2001. Antifungal Proteins. Applied and Environ. Microbiol., 67: 2883-2894.
4. Yun, D.J., Y. Zhao, J.M. Pardo, M.L. Narasimhan, B. Damsz, H. Lee, L.R. Abad, M.P. D'Urzo, P.M. Hasegawa and R.A. Bressan, 1997. Stress Proteins on the Yeast Cell Surface Determine Resistance to Osmotin, a Plant Antifungal Protein. Proceeding of National Academy, USA, 94: 7082-7087.
5. Hamood, M.S. and F.A. Fattah, 1989. Reaction of Wheat Genotypes to Infection by *Anguina tritici*. Review of Nematol., 12: 429-431.
6. Laughlin, C.W., 1998. Plant-Parasitic Nematodes and Their Management. Available on internet at: www.2.ctahr.hawaii.edu/OC/freepubs/pdf.PD-15.pdf
7. King, I.P., Forster, B.P. Law, C.C. Cant, K.A. Orford, S.E. Gorham, S.J. Reader and T.E. Miller, 1997. Introgression of salt-tolerance genes from *Thinopyrum bessarabicum* into wheat. The New Phytologist, 137: 75.
8. Behdad, A., 1996. Iranian Encyclopedia of Plant Protection, Pests, Diseases and Weeds. Yadbood Press: Esfahan, Iran.
9. Elahinia, A., 1998. Introductory Mycology and Plant Pathology. Guilan Univ. Press: Guilan, Iran.
10. Czeeh, J.G., 2002. Achievements and Prospects of Wheat Breeding for Disease Resistance. Plant Breeding, 38: 16-28.
11. Okhovat, M., 1999. Diseases of Cereal Crops. Tehran Univ. Press: Tehran, Iran.
12. Hassani, H.S., I.P. King, S.M. Reader, P.D.S. Caligari and T.E. Miller, 2000. Can *Tritipyrum*, a new salt tolerant potential Amphiploid, be a successful cereal like *Triticale*? J. Agril. Sci. Technol., 2: 177-195.
13. Shahsev and H. Hassani, 1999. New hopes for salinity resistance in the newly developed artificial wheat for arid and saline soils of Iran. First Conference on Kavir Loot of Iran, 1: 278.
14. Shahsev and H. Hassani, 2000. First complete report on development and commercial release of Allopolyploid of *Tritipyrum*: a new Amphiploid resistant to saline soils among different genera of cereal crops. Sixth Congress of Agronomy and Plant Breeding of Iran, 1: 579.
15. William, M.D.H.M. and A. Mujeeb-Kazi, 1993. *Thinopyrum bessarabicum:* biochemical and cytological markers for the detection of genetic integration in its hybrid derivatives with *Triticum aestivum* L. Theoretical and Applied Genetics, 86: 365-370.

16. Anonymous, 1995. Seed treatments and genetic resistance for Controlling Smut diseases in winter wheat. Research Annual Report. Spec. Rpt. 946. pp. 81-95. Available on internet at: http://Pwa.ars.Usda.gov/pend/eton/cpcrc/Annual/Report-Web/datafiles
17. Shahsev, H. Hassani, P.D.S. Caligari, S.M. Reader, I.P. King and T.E. Miller, 2000. Can tritipyrum, a new salt tolerant potential amphiploid, be a successful cereal like triticale? J. Agril. Sci. Technol., Islamic Republic of Iran, 2: 177-195.
18. Pest Control Note 94-08, 1998. Crop Protection Factsheet, Ministry of Agriculture, Food and Fisheries, Government of British Colombia. Available on internent at: http://www.agf.gov.bc.ca/cropprot/dbunt.htm
19. Kong, L.D., D.D. Tzeng and C.H. Yang, 2001. Generation of PCR-based DNA fragments for specific detection of *Streptomyces saraceticus* N45. Proc. Natl. Sci. Counc. Roc (B), 25: 119-127.