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Original Contribution

STUDY OF LATENT PERIOD AND INTERACTIONS BETWEEN DIFFERENT *SEPTORIA TRITICI* GENOTYPES AND DIFFERENT WHEAT CULTIVARS AND LINES IN GREENHOUSE

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ABSTRACT

In this study, the interaction of four genotypes of *Septoria tritici* -the causal agent of Septoria blotch- which were collected from Khuzestan (Safiabad), Ardebil (Moghan) and Golestan (Gorgan) provinces with (Tajan, Zagros, Koohdasht, Shiroodi, Shanghai, Falat and Darab-2) cultivars and two (N-80-6 and N-80-19) lines of wheat were examined. The factorial experiment was carried out in a compeletely random design with four replications in the greenhouse. Disease severity was measured using double digit scale from disease appearance to heading. The Area Under Disease Progress Curve (AUDPC) and disease severity was calculated and used in analysis. The results showed that there is a significant difference between these cultivars and lines, four isolates and interaction between cultivars and isolates. Also, cultivars Falat and Darab-2 had maximal and cultivar Shanghai had minimal AUDPC. Golestan province isolates had the highest AUDPC in all tested cutivars. Statistically Significant interaction of isolate-cultivar showed that virulence of each isolate (pathogen genotype) on each cultivar or line is different from other cultivars and lines. This showed that, there is a host- pathogen specificity in *S. tritici*-wheat pathosystem. The results showed that the length of latent period was varied between 16 and 20 days in different cultivars and lines and it showed a significant difference between cultivars and lines. This is the first study on length of latent period and interaction between *S. tritici* genotypes collected from three provinces with nine cultivars and lines of wheat in Iran.

Key words: Isolate- Cultivar Interaction, Latent period, *Septoria tritici*, Genotype, AUDPC.

INTRODUCTION

The Septoria blotch diseases of wheat are incited by *Septoria tritici* Roberg in Desmaz. (Telemorph: *Mycosphaerella graminicola* (Fuckel) J. Schrot in Cohn) cause major foliar disease of wheat, inflicting considerable yield losses in many countries worldwide (1). Disease importance and crop loss were significant when Mexican cultivars with good farm characters like; high yield, toleration to various environments and resistance to rust were used in many countries. It caused significant crop loss in many countries because of their susceptibility to Septoriosis (2, 3).

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Disease incidence depends on cultivar susceptibility, inoculum availability, crop management practices and favorable environmental conditions (cool temperature, high humidity and frequent rain). The greatest risk to a crop is related to the occurrence of conditions that favor spore dispersal during and shortly after flag leaf emergence. Spore dispersal and infection at this time favors a second generation of pathogens (4). Vertical spread is a key factor determining severe disease on the uppermost leaf layers of a wheat crop (5). This disease is second important disease (after yellow rust) in hot and moderate climate of Iran (6). The epidemics of this disease were occurred in most parts of Iran at 1996 (7) and in Golestan province during 2002-2003 (8). Leaf and glum wheat Septoria blotch decrease 31 to 51 percent of yield yearly (9).

Eyal *et al*., (10) reported a clear differential interaction and, therefore, clear physiological specialization of five isolates of *S. tritici* on 14 cultivars of wheat in Israel. Also, they reported the existence of physiologic specialization in *S. tritici* and stressed that in the *S. tritici*–wheat pathosystem, host response is quantitative (% necrosis, % pycnidia coverage) rather than qualitative. However, most investigations report quantitative differences in pathogenicity of isolates of *S. tritici* and, hence, indications of a certain degree of physiological specialization of these isolates to particular wheat cultivars (11, 12). Due to the quantitative aspects of the interaction between *S. tritici* and the hosts, race designation has been avoided in most investigations; one except being Saadaoui (12) who designated three races in Morocco (13). A study was conducted to identify genotypic differences in the response of winter wheat to artificial inoculation with several *S. tritici* populations. Significant effects of host genotype, pathogen population and host- pathogen interaction were found (14). Variation in virulence patterns within and between populations of *S. tritici* was shown by assessing host response (mostly % pycnidial coverage) on a selected set of cultivars, with little commonality apparently between results obtained wheat genotypes composing the differential set of cultivars (15). Knowing the length of the latent period under different environmental conditions assists in identifying the conditions or events that caused the infection (16). Furthermore, length of latent period is an important epidemiological factor in the subsequent spread of the pathogen within and beyond the crop (17). The length of the latent period depends on cultivar and environmental conditions, such as temperature and leaf wetness, and has been reported to vary from 14 to 21 days at the optimum temperature (15-20°C) to 40 days at 5°C (9, 18). Long latent periods also mean that disease control measures may be necessary some time before serious symptoms occur (19).

This is the first study on length of latent period and interaction between *S. tritici* genotypes collected from three provinces with nine cultivars and lines of wheat in Iran.

MATERIALS AND METHODS

Fungi isolation. Green leaves with pycnidia of septoria were collected from different regions including; Golestan (Gorgan), Ardebil (Moghan) and Khuzestan (Safiabad) provinces. Isolation of the fungi was performed by Eyal *et al*. (9) method and purified by single spore method (9).

Molecular studies. To detect and molecular identification of pathogen in infected plants species- specific primers were used (20). The below.

ITS1: 5' TCCGTAGGTGAACCTGCGG 3'
JB446: 5' TCCTCCGCTTATTGATATGC 3'

Mycelial biomass of each isolates was powdered in liquid nitrogen and DNA extraction was performed according to the protocol described by Safaie *et al*. (21). Quality and quantity of genomic DNA was examined by biophotometer and 0.8% agarose gel. To extract DNA from infected and healthy leaves, freezed leaves of each wheat cultivars and lines were powdered in liquid nitrogen and suspended in 400 µl TBE buffer (Tris 90 mM, Boric acid 90 Mm and EDTA 2 mM) immediately. Then microtubes were stored at 96°C for 10 minutes and then placed in ice for 5 minutes. At last they centrifuged at 4°C for 10 minutes by 13000rpm and the upper liquid layer was used in PCR. PCR reactions were performed in a volume of 20 µl (21). Reactions were run for one cycle, 2 min at 94°C and 30 cycles, each consisting of 15 s at 94°C, 15 s at 56°C and 45 s at 72°C (20) in a Ependorf Gradient thermocycler. The products were analyzed by electrophoresis of 10µl aliquot of each PCR sample on 0.8-1% Horizontal agarose gel.

Cultivar- isolates interaction. The interaction of four genotypes of *S. tritici* with Tajan, Zagros, Koohdasht, Shiroodi, Shanghai, Falat and Darab-2 cultivars and N-80-6 and N-80-19 lines were examined. Factorial experiment was carried out in a completely random design with four replications in the greenhouse. Artificial inoculation was done twice. One replication without artificial inoculation was considered as control. Potato dextrose broth was inoculated with 5-mm plug of each fungal isolate and was shaken for 4-7 days at 25°C. Spore concentration was adjusted to 2×10^6 spores/ ml. Seedlings were inoculated at the two- leaf stage using the quantitative techniques of Eyal *et al.* (9). After inoculation pots were covered with transparent plastic for 72 hours to increase humidity and promote infection.

Disease severity was assessed 15 days after leaves; using the Saari- Prescott (22) scale. Also disease severity was recorded (9, 23). Disease recordings were continued until flowering. Area under Disease Progress Curve (AUDPC) was calculated (24).

$$\text{AUDPC} = \sum_{i}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

The experiment was repeated two times. Variance analyses were done using MSTATC software.

Latent period. To determine the length of latent period in different cultivars and lines, the time interval from inoculation until pycnidia appeared was recorded in each cultivar and inoculation on the first (coleoptilar) and second line. Data were analyzed using MSTATC software.

RESULTS AND DISCUSSION

Molecular studies. In order to investigate the specificity of *S. tritici* specific primers determined by Beck and Ligon (20), we examined weather they produce PCR products from other fungal pathogens of wheat such as *Fusarium graminearum*. The *S. tritici* specific primers (ITS1 and JB446) did produce PCR products only from this pathogen revealing that these primers can be used to diagnose *S. tritici* isolates **(Figure 1A)**. Also these primers can detect *S. tritici* in infected plant tissue as well **(Figure 1B)**.

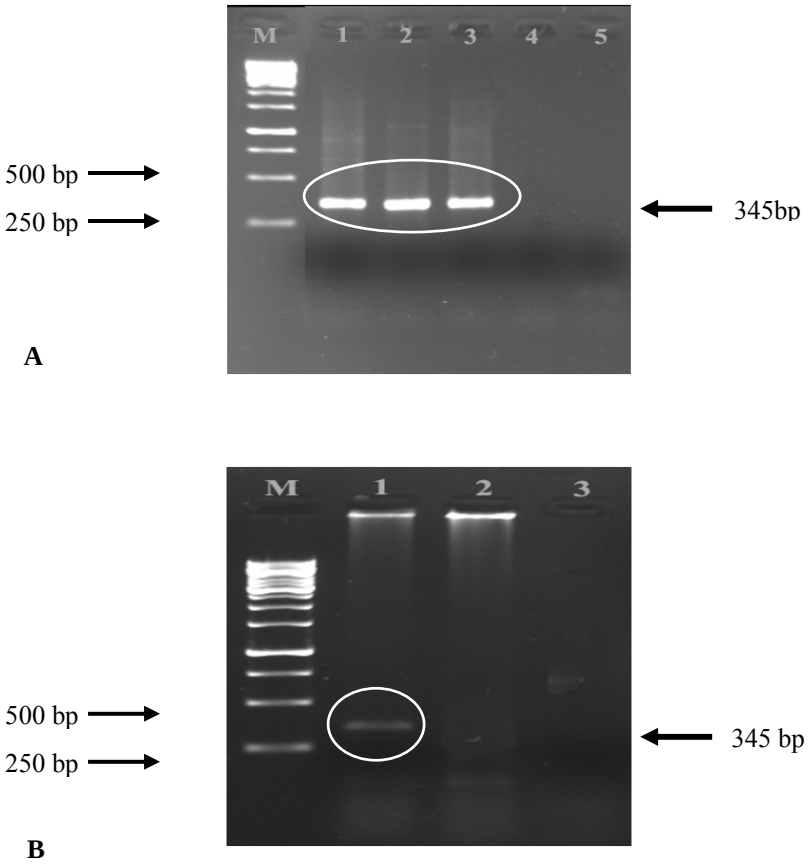

Figure 1. *Ethidium bromide stained agarose gel of polymerase chain reaction products using ITS1 and JB446 primers.* **A**: *M: Gene ruler 100bp DNA ladder, 1, 2 and 3: Septoria tritici isolates, 4: Fusarium graminearum and 5: negative control.* **B**: *M: Gene ruler 100bp DNA ladder, 1: infected wheat, 2: healthy wheat and 3: negative control.*

Latent period. The study of latent period in greenhouse showed that there is a significant difference (P≤0.01) between cultivars and lines **(Table 1 and Figure 2)**. The length of latent period was varied between 16 and 20 days in different cultivars and lines **(Figure 2)**. Our results were corresponding to Eyal *et al*. (9) and Shaw (18). Greenhouse experiment was repeated twice and both results were the same.

Table 1. *Variance analyses of latent period of Septoria tritici in Tajan, Zagros, Shiroodi, Koohdasht, Shanghai cultivars and N-80-6, N-80-19 lines in greenhouse*

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Replication	3	0.000	0.000	0.000
Cultivar/Line	6	94.86	15.81	35.571*
Error	18	8	0.44	
Total	27	102.86		

* P≤ 0.01, Coefficient of variation= 3.79%

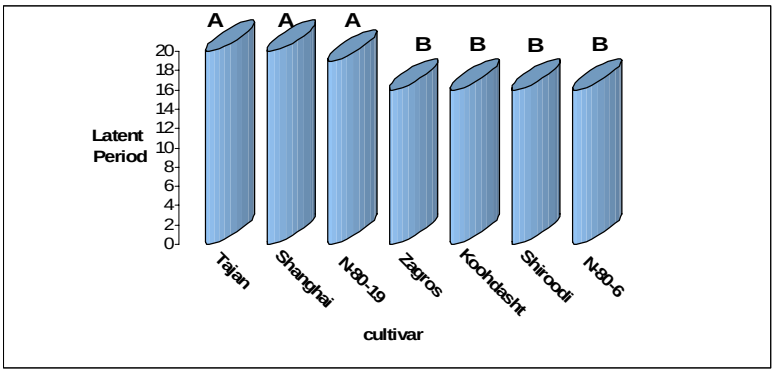

Figure 2. *Comparison of means of latent period of Septoria tritici in different wheat cultivars (Tajan, Zagros, Shiroodi, Koohdasht, Shanghai) and lines (N-80-6, N-80-19) in greenhouse*

Cultivar- isolates interaction. The Area under Disease Progress Curve (AUDPC) data's showed that there was a significant difference among cultivars and lines, four isolates and interaction between cultivars and isolates (Table2). This was Due to different host genotypes and different Response of host-pathogen against pathogen (14). Variance analyses of cultivar- isolate interaction and different isolates were showed at **table2**, **figure3 and 4**, respectively. Also results showed that cvs. Drab-2, Falat and Koohdasht had the highest disease incidence against different isolates, respectively and Shanghai had the lowest one among all tested cultivars. Based on disease progress in greenhouse and field, Shanghai revealed more resistance compared with other cultivars. Consequently the lowest AUDPC was acceptable. These experiments were repeated twice and both of them were the same.

Table 2. *Variance analyses of Area under Disease Progress Curve in cultivars (Tajan, Zagros, Shiroodi, Koohdasht, Shanghai) and lines (N-80-6, N-80-19) interact with four genotypes of Septoria tritici in greenhouse*

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Replication	2	24348.98	12174.49	0.79
Isolate	3	594986.17	198328.73	12.79*
Cultivar/Line	8	3030148.37	378768.55	24.42*
Cultivar-Isolate	24	841206.75	35050.28	2.26*
Error	70	1085701.86	15510.03	
Total	107	5576392.13		

* P≤ 0.01, Coefficient of variation= 17.13%

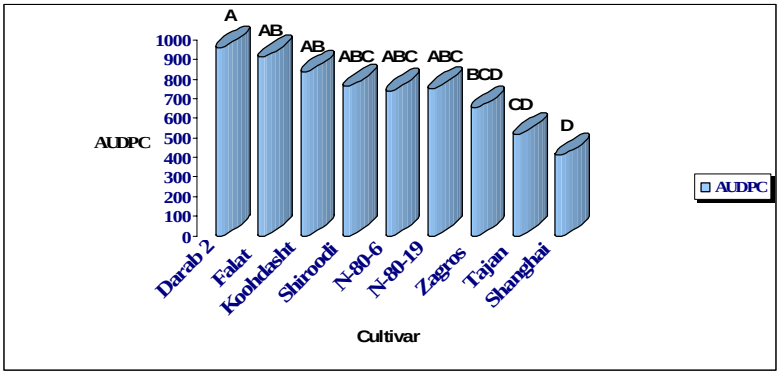

Figure 3. *Comparison of means of Area under Disease Progress Curve in different wheat cultivars (Tajan, Zagros, Shiroodi, Koohdasht, Shanghai) and lines (N-80-6, N-80-19) in interaction with four genotypes of Septoria tritici*

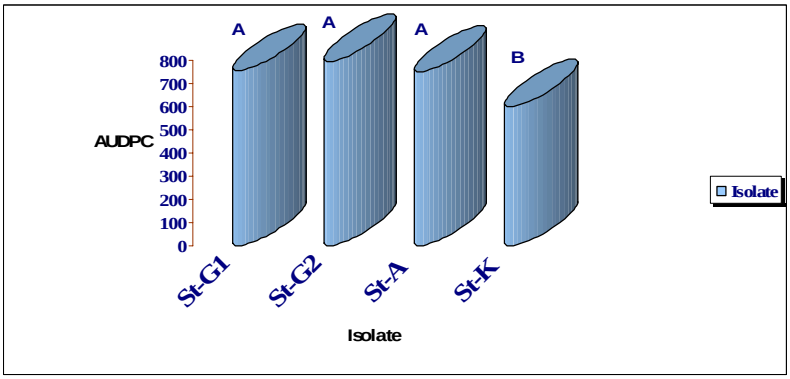

Figure 4. *Comparison of means of Area under Disease Progress Curve of four Septoria tritici genotypes (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate)*

The resistance slows the arte of host colonization but has no appreciable effect on the process of lesion development (25). Consequently, the lowest AUDPC in Shanghai cultivar was acceptable. A statistically significant difference ($p<0.01$) also existed in interaction between isolate-cultivar indicating that virulence of each isolate (pathogen genotype) on each cultivar or line was different from other cultivars and lines, consequently supporting the idea that there is a host-pathogen specificity in *S. tritici*- wheat pathosystem. Our results were corresponding to other researchers (10, 13). This relation in *S. tritici*- wheat pathosystem emphasis that genetic structure of pathogen should be considered in wheat breeding for resistance to introduce durable resistance sources.

The interaction between each cultivar and line and four genotypes of *S. tritici* showed at **table 3 and figure 5 to 13**.

Table 3. *Area under Disease Progress Curve of Tajan, Zagros, Shiroodi, Koohdasht, Shanghai cultivars and N-80-6, N-80-19 lines interact with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate)*

Fugal	**Wheat cultivars and lines**								
isolates	Tajan	Zagros	Koohdasht	Shiroodi	Shanghai	Falat	Darab2	N-80-6	N-80-19
St-G1	A	B	A	A	A	AB	A	AB	A
St-G2	A	A	AB	B	B	B	B	A	B
St-K	B	B	B	AB	A	A	B	B	A
St- A	A	B	A	B	A	B	AB	AB	AB

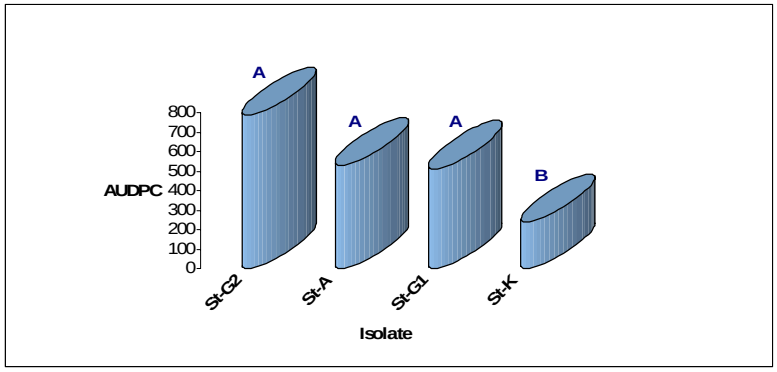

Figure 5. *Comparison of means of Area under Disease Progress Curve of wheat cv.Tajan inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

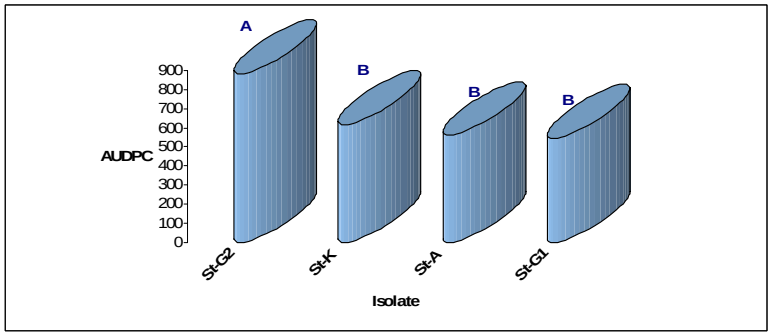

Figure 6. *Comparison of means of Area under Disease Progress Curve of wheat cv. Zagros inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

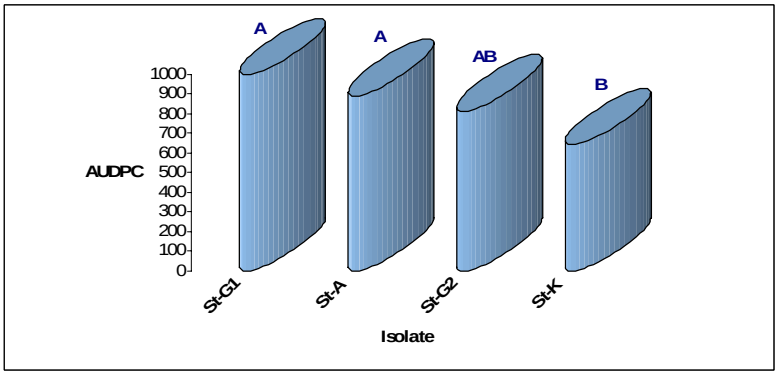

Figure 7. *Comparison of means of Area under Disease Progress Curve of wheat cv. Koohdasht inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

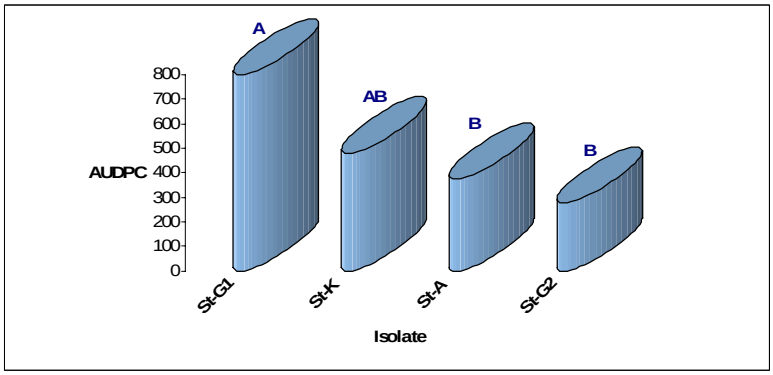

Figure 8. *Comparison of means of Area under Disease Progress Curve of wheat cv. Shiroodi inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

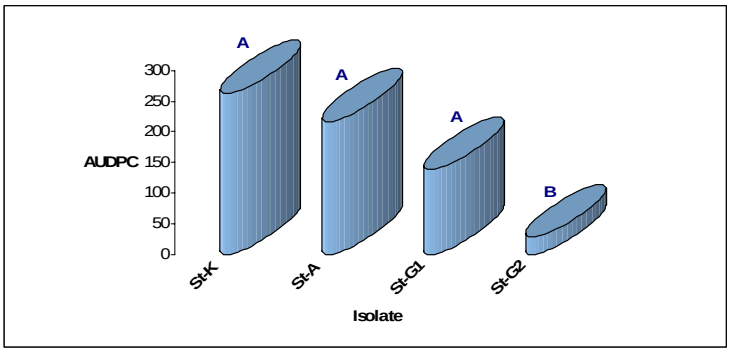

Figure 9. *Comparison of means of Area under Disease Progress Curve of wheat cv. Shanghai inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

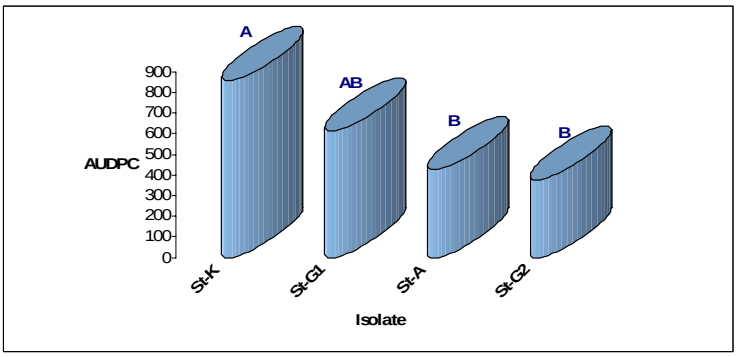

Figure 10. *Comparison of means of Area under Disease Progress Curve of wheat cv. Falat inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

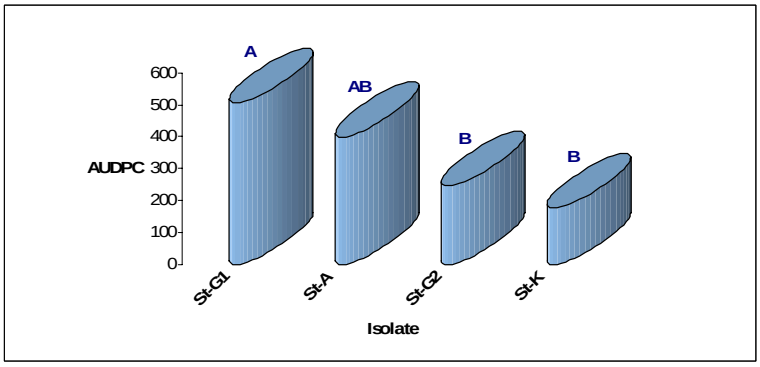

Figure 11. *Comparison of means of Area under Disease Progress Curve of wheat cv. Darab- 2 inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

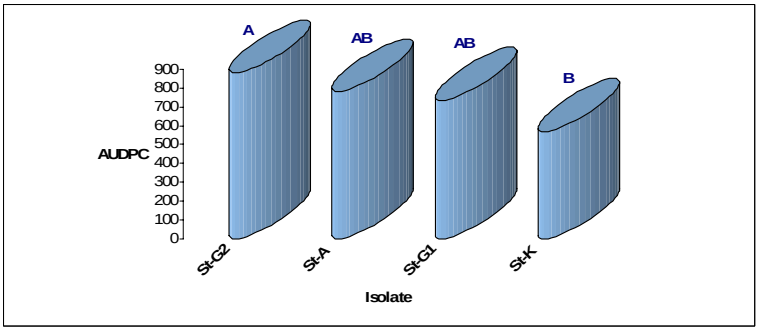

Figure 12. *Comparison of means of Area under Disease Progress Curve of wheat N-80-6 line inoculated with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

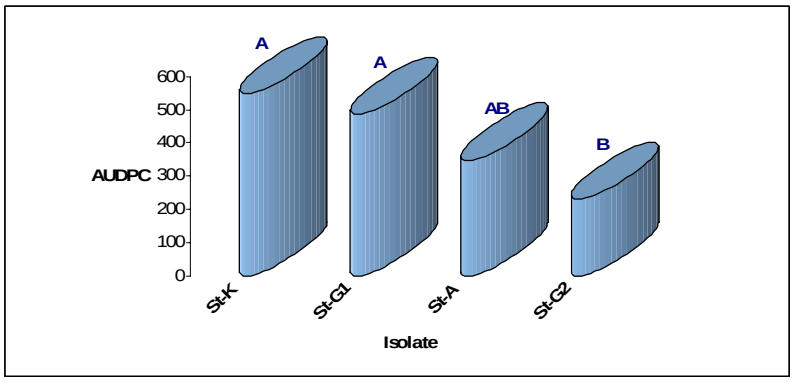

Figure 13. *Comparison of means of Area under Disease Progress Curve of wheat N-80-19 line interaction with four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate).*

The results revealed that interaction of each cultivar and line with each genotype of *S. tritici* was differed from other genotype. Golestan province isolates had the highest AUDPC in all tested cultivars. The interaction of each genotype with various cultivars and lines showed at **table 4** and **figure 14 to 17**. Darab-2 and Falat revealed the highest susceptibility against different genotypes.

Table 4. *Area under Disease Progress Curve of four genotypes of Septoria tritici (St- G1 and St- G2, Golestan isolates, St- K, Khuzestan isolate and St- A, Ardebil isolate) interact with Tajan, Zagros, Shiroodi, Koohdasht, Shanghai cultivars and N-80-6, N-80-19 lines*

Wheat cultivars	**Fungal isolates**			
and lines	St-G1	St-G2	St-K	St- A
Tajan	CD	AB	C	CD
Zagros	CD	AB	AB	BCD
Koohdasht	AB	AB	A	A
Shiroodi	BC	BC	A	ABC
Shanghai	D	C	BC	D
Falat	AB	A	A	AB
Darab2	A	AB	A	A
N-80-6	BC	AB	ABC	ABC
N-80-19	BC	AB	ABC	A

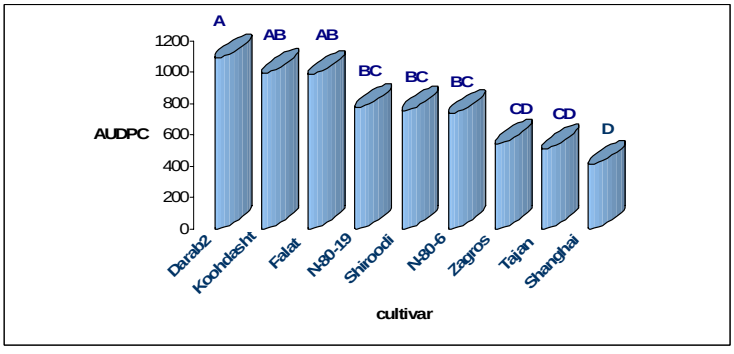

Figure 14. *Comparison of means of Area under Disease Progress Curve of St- G1 (Golestan province) isolate of Septoria tritici interaction with different wheat cultivars (Tajan, Zagros, Shiroodi, Koohdasht, Shanghai) and lines (N-80-6, N-80-19)*

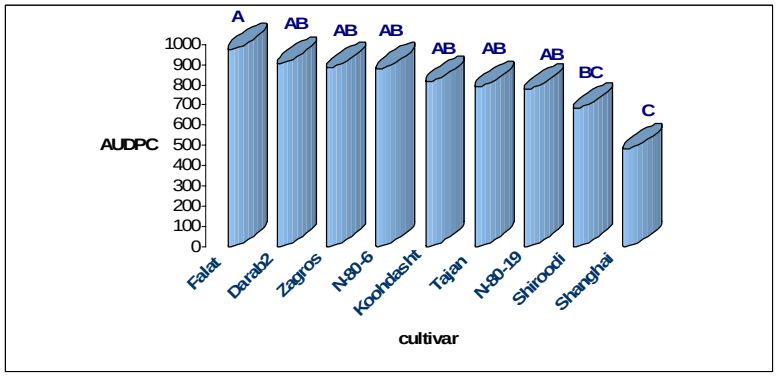

Figure 15. *Comparison of means of Area under Disease Progress Curve of St- G2 (Golestan province) isolate of Septoria tritici interaction with different wheat cultivars (Tajan, Zagros, Shiroodi, Koohdasht, Shanghai) and lines (N-80-6, N-80-19)*

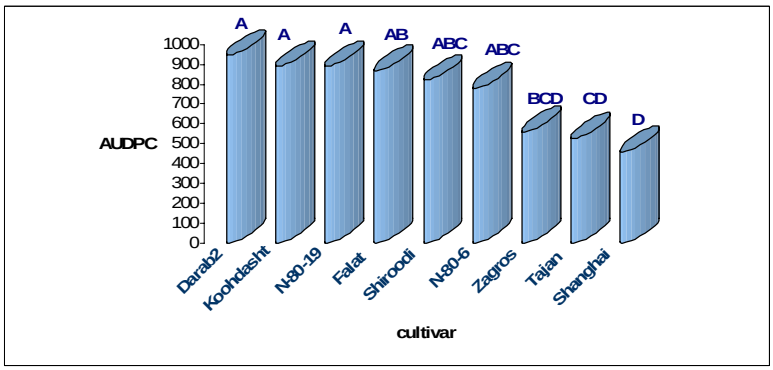

Figure 16. *Area under Disease Progress Curve of St- A (Ardebil province) isolate of Septoria tritici interaction with different wheat cultivars (Tajan, Zagros, Shiroodi, Koohdasht, Shanghai) and lines (N-80-6, N-80-19)*

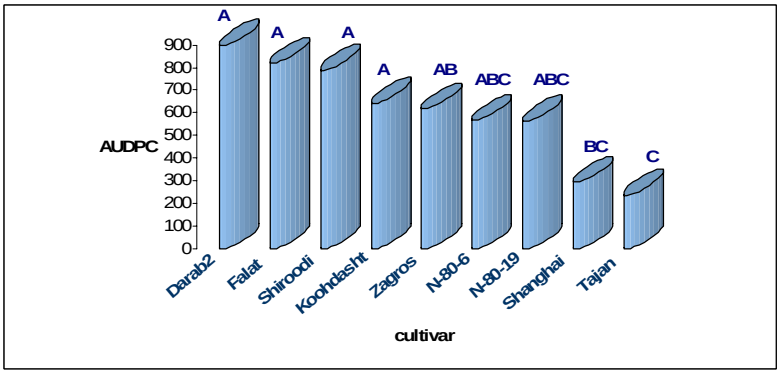

Figure 17. *Area under Disease Progress Curve of St- K (Khuzestan province) isolate of Septoria tritici interaction with different wheat cultivars (Tajan, Zagros, Shiroodi, Koohdasht, Shanghai) and lines (N-80-6, N-80-19)*

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