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https://doi.org/10.1590/SciELOPreprints.5490

Submitted on: 2023-01-24
Posted on: 2023-01-31 (version 1)
(YYYY-MM-DD)

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Estabelecimento e sanidade de plântulas de trigo produzidas a partir de sementes infectadas com *Pyricularia oryzae* Triticum

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ABSTRACT

Pyricularia oryzae Triticum (PoT) is known to survive in wheat seeds and this condition favors its spread. There are, however, many gaps in knowledge regarding the damage caused by this pathogen in relation to the establishment and sanity of wheat seedlings. The objective of this study was to determine whether there is a correlation between incidence of PoT in wheat seeds and variables associated with the establishment and the sanity of the produced seedlings. Seven samples of wheat seeds naturally infected with the pathogen were used. The incidence of PoT in each of the seed samples was determined via the Blotter test, indicating a variation from 0 to 80.5%. The seeds of each sample were sown in 500 mL plastic pots containing autoclaved sand and the seedlings produced were evaluated in relation to the following aspects; emergency, height, lesions in different segments, mortality, abnormalities and transmissibility of symptoms. The study demonstrated that there is a correlation between incidence of PoT in wheat seeds and the following variables related to the seedlings produced; height at 7 days after sowing (DAS), incidence and severity of symptoms at 7, 14 and 21 DAS, and mortality at 14 and 21 DAS. The results confirmed that the establishment and sanity of wheat seedlings directly depend on incidence of PoT in the seeds.

Key words: wheat blast, inoculum source, seedling death, transmissibility of symptoms.

RESUMO

Sabe-se que *Pyricularia oryzae* Triticum (PoT) sobrevive nas sementes de trigo e esta condição favorece sua disseminação. Há, porém, muitas lacunas no conhecimento relativo aos danos causados por este patógeno em relação ao estabelecimento e à sanidade das plântulas de trigo. O objetivo do trabalho foi verificar se existe correlação entre incidência de PoT em sementes de trigo e variáveis associadas ao estabelecimento e à sanidade das plântulas geradas. Foram utilizadas sete amostras de sementes de trigo naturalmente infectadas com o patógeno. A incidência de PoT em cada uma das amostras de sementes foi determinada via *Blotter test* indicando variação de 0 a 80,5%. As sementes de cada amostra foram semeadas em copos plásticos de 500 mL contendo areia autoclavada e as plântulas produzidas foram avaliadas em relação aos seguintes aspectos; emergência, altura, lesões em diferentes segmentos, mortalidade, anormalidades e transmissibilidade de sintomas. O estudo demonstrou que existe correlação entre a incidência de PoT em sementes de trigo e as seguintes variáveis relativas às plântulas produzidas; altura aos 7 dias após a semeadura (DAS), incidência e severidade de sintomas aos 7, 14 e 21 DAS, e mortalidade aos 14 e 21 DAS. Os resultados confirmaram que o estabelecimento e a sanidade das plântulas de trigo dependem diretamente da incidência de PoT nas sementes.

Palavras-chave: brusone do trigo, fonte de inóculo, morte de plântulas, transmissibilidade de sintomas.

INTRODUCTION

Currently, blast control is considered one of the main phytosanitary challenges to wheat production in the world. Wheat blast is a disease caused by the Triticum pathotype of the fungus *Pyricularia oryzae* Cavara (PoT) (synonym *Magnaporthe* *oryzae* B. C. Couch) (COUCH & KOHN, 2002; CASTROAGUDÍN et al., 2017; MARTINEZ, et al. 2021). Despite affecting the whole aerial part of the wheat plant, the most common symptom in the fields caused by this disease is the total or partial bleaching of spikes (URASHIMA et al., 2004; CRUZ et al., 2015). This symptom is caused by the infection of the fungus in the rachis of the spikes, which causes interruption of photoassimilate transport, affecting grain filling, making them small, wrinkled and with low specific weight (GOULART et al., 2007). Although aerial dispersion of PoT spores plays a very relevant role in the development of the disease (URASHIMA et al., 2007; DANELLI et al., 2019), it is likely that the propagation of the pathogen from infected seeds and grains (MACIEL et al., 2014; RODRIGUES, et al., 2020; TEMBO et al., 2020; VALENT et al., 2021) has played the main role in its distribution in Brazil, South America and the world. In this context, the first record of occurrence of PoT in Bangladesh and India has been attributed to the export of infected wheat grains from Brazil (CRUZ & VALENT, 2017). Furthermore, phylogenetic analyzes of PoT isolates collected in Bangladesh indicated genetic similarity with isolates that have caused epidemics in South America (ISLAM et al., 2016; MALAKER et al., 2016; CERESINI et al., 2018). In Brazil, the use of seeds infected with PoT at sowing was the probable cause of the spread of the fungus in wheat from the Brazilian states of Paraná to Mato Grosso do Sul (URASHIMA et al., 2007) and, in triticale, from Paraná to São Paulo (MEDINA et al., 2009).

The transmission of PoT by wheat seed has already been studied by GOULART & PAIVA (1990). More recently, GOMES et al. (2018) carried out a very detailed evaluation on the subject, having concluded that the rate of PoT transmission from seed to seedlings, in experiments carried out under controlled conditions, is low. The study carried out by GOMES et al. (2018) constitutes an important contribution to the theme, however the lack of definition about the exact infection rate of PoT in the seeds used in the experiments greatly limited the possibility of establishing more precise relationships between initial condition of the seeds, in relation to the pathogen incidence, and consequences of its action, represented by the implications in terms of emergence and sanity of seedlings.

According to ISLAM et al. (2016), better understanding all aspects related to PoT survival is an important condition for establishing disease management strategies in order to mitigate or reduce the harmful effects caused by blast. In this sense, VALENT et al. (2021) states that more knowledge about the transmissibility of PoT from seeds infected with this pathogen shall contribute to the definition of disease control strategies. We emphasize that the detection, identification and effects caused by causal agents associated with seed plant diseases are considered fundamental issues for formulation of management strategies. However, we understand that evaluations and studies related to the transmissibility of PoT under controlled conditions should use seeds with broader or more comprehensive pathogen infection rates than what has been used in previous works (GOULART & PAIVA, 1990; GOMES et al., 2018), a condition that must facilitate the establishment of more assertive and precise regression equations for the relationship between incidence of the pathogen in wheat seeds and response in produced seedlings.

The objective of this study was to determine whether there is correlation between incidence of PoT in wheat seeds and variables associated with establishment and sanity of produced seedlings.

MATERIALS AND METHODS

The experiments were carried out in facilities such as incubation chamber and plant pathology laboratory belonging to Embrapa Wheat, a unit of the Brazilian Agricultural Research Corporation (Embrapa), Passo Fundo, Rio Grande do Sul (RS), Brazil. Samples containing 300 g of seeds were obtained from wheat fields cultivated in 2019 in different municipalities in Brazil. They were classified according to their origin (Table 1) as being from a) RS, samples A1, A2, A3 and A7; and b) Central Brazil, samples A4, A5 and A6.

The incidence of PoT in the seeds was performed using the blotter test method (BRASIL, 2009). The seeds were distributed in Gerbox-type acrylic boxes, containing two sheets of blotting paper soaked in distilled and sterilized water. Two hundred seeds taken at random from each sample were analyzed. The seeds were distributed equidistantly, in number of 25 per box, and incubated in a controlled environment (25 °C and photoperiod of 12 h light/12 h dark) for seven days. After this period, they were observed individually with the aid of a stereomicroscope and an optical microscope. Presence of PoT was confirmed based on occurrence of reproductive structures typical of the pathogen on seeds, data which were transformed into percentage incidence of the pathogen in each sample (BRASIL, 2009). After that, 120 undisinfected seeds from each sample were sown in 500 mL plastic pots containing sieved autoclaved sand (120 °C; 1 atm; 1 h) as substrate. Twenty pots were used per sample, and each experimental unit (pot) received six seeds. The experiments were conducted in a completely randomized design in a controlled environment chamber with temperature and relative humidity ranging from 24 to 27 °C and from 65 to 75%, respectively. Fertilization was carried out ten days after sowing (DAS) using 500 mL of nutrient solution (HOAGLAND & ARNON, 1950) in a fertirrigation system.

The variables measured in the experiments were the followings: a) seedling emergence at 7 DAS; b) seedling height at 7, 14 and 21 DAS (measurements taken from the soil surface in the pot to the extended tip of the tallest leaf); c) incidence of blast symptoms in seedlings at 7, 14 and 21 DAS (obtained according to the frequency of symptomatic coleoptile, caulicle or primary leaves); d) severity of lesions on the roots, mesocotyls and primary leaves at 21 DAS (obtained through means of visual estimation of the symptomatic area); e) percentage of dead plants at 7 and 14 DAS; f) percentage of abnormal seedlings at 14 DAS (obtained from those with atrophied hypocotyl with a thick and short appearance, stunted main root and/or twisted hypocotyl); g) rate of transmissibility of symptoms from seed to seedling at 7, 14 and 21 DAS.

The transmissibility of PoT from seeds to wheat seedlings was obtained according to the formula described by FORCELINI (1991), that is, by determining the percentage ratio between symptomatic plants, that is, with incidence of lesions in the aerial part, whether in coleoptiles, stems or primary leaves, in relation to the rates of initial infection of the pathogen in the seeds.

$$\text{Rate of transmissibility of symptoms (\%)} = \frac{\text{Symptomatic seedlings (\%)}}{\text{Initial seed infection (\%)}} \times 100$$

The data obtained were subjected to analysis of variance (ANOVA) and the means were compared by Tukey's test at 5% error probability, with the aid of the R software (R DEVELOPMENT CORE TEAM, 2017). The transmissibility rate variable was subjected to analysis of mixed effect models for intercept and factors. With the aid of the Windows Excel program, the following information and/or statistical data were obtained: trend lines, linear regression equations, and coefficients of correlation (r) between incidence of PoT in wheat seeds and variables associated with establishment and sanity of produced seedlings. The significance of r was evaluated with appropriate critical values of Student *t*-distribution for significance of 0.01 and 0.05 (SCHABENBERGER & GOTWAY, 2005).

RESULTS

The incidence of PoT in the samples collected in Rio Grande do Sul (A1, A2 and A7) ranged from 0 to 13%, and from 22.8 to 80.5% in the samples collected in the Central Brazil (A3, A4, A5 and A6; Distrito Federal and Goiás) (Table 1). There was difference between the samples in relation to all the variables evaluated in the study (Table 2).

The seedling emergence rate ranged from 54.5 to 97.7% (Table 2). There was difference between the samples regarding this variable with the formation of four statistical groups. The highest seedling emergency rate was observed in the sample A7 (initial incidence of PoT was 0.0%), although there was no statistical difference between this sample and A3.

The sample with the lowest emergency rate was A6. The height of seedlings in sample A7 was classified into groups of greater height in the three evaluations carried out during the experiment (Table 2). Sample A1 and A5 were classified into the same groups as A7 at 7 and 21 DAS, respectively. The smallest height observed was in the sample A6, and this condition was observed in the three evaluations performed.

The incidence of symptoms was evaluated in coleoptiles, stems and primary leaves (Table 2 and Figure 1). In the three evaluations carried out, no symptoms were observed in the sample A7. At 7 DAS, with the exception of A7, symptoms were already observed in the seedlings of all samples. On the other hand, the sample A6 was the one that presented the highest PoT occurrence in the three evaluations. With the exception of sample A7, the incidence of symptoms in seedlings increased throughout the evaluations in all samples with positive PoT infection. The evaluation of the severity of symptoms on the roots, mesocotyls and primary leaves of the seedlings at 21 DAS (Table 2) was done with the seedlings having been carefully extracted from the pots with sand and washed in running water. These circumstances allowed the symptoms to be evaluated more easily, estimating severity with greater precision. There was a difference in relation to this variable between the samples in the three types of seedling segments, but with no symptoms in sample A7. Among the three evaluated plant segments, it was in the roots that the symptoms were least developed.

At 14 DAS, no dead plant was found in sample A7 and the highest percentage of mortality occurred in sample A6 (5.4%) (Table 2). At 21 DAS, the highest mortality rate was also observed in the A6 sample (48.2%) and 4.8% mortality rate was also observed in the A7 seedlings. There was an increase in the mortality rate of all samples between the two evaluations performed. Furthermore, with the exception of sample A7 (with 0% incidence of PoT detected in the blotter test), at 21 DAS, all mortality rates observed in the samples were relatively high, with rates above 13%.

As for the occurrence of abnormal seedlings (Table 2), the highest rate at 14 DAS was observed in the A6, with 2.9%, however, this did not differ statistically from samples A2 and A5. The lowest percentage of abnormal seedlings was 0.6% in the sample A1, which did not differ statistically from the A7. Compared with the other variables evaluated in the experiments, the occurrence of abnormal seedlings was one that presented the lowest absolute values, regardless of the incidence of PoT detected in the blotter test.

As there was no incidence of symptoms in the seedlings of the A7 sample, there was also no transmissibility in the seedlings of these samples. At 7 DAS, the difference between samples was relatively low, ranging from 3.5 to 15.0% (Table 2). In this evaluation, the samples A1, A4 and A6 presented higher transmissibility rates than the other samples. The lowest transmissibility rate was observed in the A2, which was 3.5%. There was a significant increase in the values of this variable throughout the evaluations, with means increasing from 11.3 to 37.0% and then to 63.4% at 7, 14 and 21 DAS, respectively. The values presented by the sample A6 demonstrate this evolution of transmissibility quite well throughout the evaluations, going from 13.3 to 34.0% and then to 91.2%, at 7, 14 and 21 D.A.S, respectively. It was found that, even in the sample A6, with an incidence of PoT of 80.5% in the seeds, there was no transmissibility of symptoms to 100% of the seedlings, even though the highest percentage of dead seedlings was registered in this sample.

The symptoms observed in the seedlings were in accordance with what is reported in the literature (Figure 1; URASHIMA et al., 2009; MACIEL et al., 2014). In the lesions of the primary leaves, the main symptoms were spots, generally elliptical or rounded, with a dark brown margin and grayish center. In the mesocotyl region, there was also an incidence of very characteristic elliptical spots. In the roots, it was observed that they became dark and necrotic.

No correlation was observed between the incidence of PoT in the seeds and emergence at 7 DAS, as well as in relation to seedling height at 14 and 21 DAS, seedling abnormality at 14 DAS, and the transmissibility of symptoms at 7, 14 and 21 DAS (Table 3). On the other hand, a correlation was found between the incidence of PoT in the seeds and the following variables: seedling height at 7 DAS, $r = -0.8530$; incidence of symptoms at 7, 14, and 21 DAS, $r = 0.9831$, 0.9793, and 0.9962, respectively; symptom severity at 7, 14, and 21 DAS, $r = 0.9731$, 0.9585, and 0.9446, respectively; and mortality at 14 and 21 DAS, $r = 0.8147$ and 0.8459, respectively.

Among the correlations with the incidence of PoT in the seeds, it is important to highlight those that were established with the variables in which the incidence and severity of symptoms were evaluated, which had levels of statistical probability greater than 0.99 (Figure 2).

DISCUSSION

The confirmation of the correlation between the incidence of PoT in wheat seeds in nine of the 16 variables evaluated in the present study (Table 3) provides very consistent evidence of the existence of a quantitative pattern of reactions or responses in seedlings that is proportional to the infection rate of this pathogen in seeds. This response pattern, confirmed by linear equations with 0.95 and/or 0.99 of statistical probability, have considerable potential to be used in studies aimed at projecting or forecasting damage caused by wheat blast in fields sown with seeds infected with PoT. The fact that the results were obtained from a set of seed samples with a wide incidence rate of the pathogen (0.0 to 80.5%) increases the possibilities of precision and assertiveness in the results obtained and equations established, considering that similar works carried out previously made use of seed samples with a maximum of 40% infection rate with PoT (GOMES et al., 2018).

It was confirmed that the PoT infection in the seeds promoted harmful effects to the establishment and sanity of the seedlings. Such effects caused damage to the seedlings, which were represented by the reduction of emergence and height, lesions in different segments, mortality, abnormalities and transmissibility of symptoms. The confirmation that these effects were actually caused by PoT infecting the seeds is based on the comparison of the mean values recorded for sample A7, which did not have PoT infection, in relation to the values observed in the other samples. However, there were some exceptions in which some samples showed performance similar to A7 in relation to the variables evaluated in the experiments. It is possible to list some examples that this may have actually occurred, highlighting the following cases: incidence of symptoms at 7 DAS, A1 and A2; percentage of dead seedlings at 21 DAS, A2 and A3; percentage of abnormal seedlings at 14 DAS, A1, A3 and A4; severity on the roots at 21 DAS; TO 1; severity on primary leaves at 21 DAS, A1. Observing this list of situations, it appears that the predominant samples in terms of performance similar to sample A7 are A1 and A2, which had the lowest rates of PoT infection. This condition, possibly, was decisive for less damage to the seedlings, to the point of enabling some kind of balance or resemblance between the effects caused by the PoT infection in the samples A1 and A2 in relation to the observations collected in the sample A7.

The effect caused by the incidence of PoT in the wheat seeds in relation to the seedling abnormality variable was somewhat different from that described above for the other variables. This situation was more evident in the sample A2 which, although it had a relatively low incidence rate of PoT (13.5%), had an abnormality rate that was statistically equal to the samples with the highest incidence rates of the fungus (A5 and A6). It is possible to speculate two possible causes for this effect: a) The fact that it is a variable whose all sample means showed little variation (0.4 to 2.9%), a condition that produces limitations to differentiate them from each other, and b) the occurrence of abnormalities in the seedlings may not be a dependent effect or associated with the presence of PoT in the seeds.

It was not possible to use samples from a single cultivar in conducting the experiments due to the unavailability of seed samples from a single cultivar naturally infected by PoT and with variations in the pathogen infection rates equal to or similar to those of the samples evaluated in the study (ranging from 0.0 to 80.5%). We prioritized carrying out the study using seeds naturally infected with the pathogen because we understand that this approach is the closest to the reality faced by wheat producers in Brazil. We also understand that it is very difficult to even speculate whether or how much the "cultivar" factor may have influenced the results obtained in the study. However, we understand that the correlation observed between the incidence of PoT in the seeds and nine of the evaluated variables represents an important indication that the response pattern in seedlings is strongly associated with the pathogen infection rates in seeds, regardless of the cultivar to which the seeds belonged.

Currently, the tolerance for PoT infection in wheat seeds certified in Brazil is 10% (ABRATES, 1992; GOULART, 1995). It is possible to speculate a connection between this level of tolerance established by Brazilian legislation and the results obtained in the present study. This connection is related to the results from the samples with lower PoT infection used in the experiments, that is, samples A1 and A2, with infection rates of 8.0% and 13.5%, respectively. Given this context, it is considered important to associate this condition with the work carried out by GOULART & PAIVA (1990), who were able to very efficiently control the action of PoT as a pathogenic agent of wheat seeds with pathogen infection rates of up to 21% by treating the seeds with fungicides.

CONCLUSION

There is correlation between the incidence of PoT in wheat seeds and the following variables related to seedlings; height at 7 DAS, incidence and severity of symptoms at 7, 14, and 21 DAS, and mortality at 14 and 21 DAS.

ACKNOWLEDGEMENTS

We are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing scholarships for the second (doctoral-CAPES- 88882.427632/2019-01) and fourth (EncBolsasEMBRAPA2019- CNPq- 380657/2020-1) authors. We also thank EMBRAPA for the financial support, which was made available within the budget of project SEG 12.16.04.009.00.00.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

JLNM, ANS and IFDC conceived and designed experiments. ANS carried out the lab and statistical analyses of experimental data. JLNM and ANS prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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Table 1. Origin and cultivar of wheat seed samples collected and used in the study.

Sample	Wheat Cultivar	Municipality	Brazilian State
A1	Tbio Audaz	Butiá	Rio Grande do Sul
A2	Tbio Sossego	São Luiz Gonzaga	Rio Grande do Sul
A3	Tbio Sintonia	São Gabriel	Goiás
A4	Tbio Toruk	Cristalina	Goiás
A5	BRS 404	Planaltina	Distrito Federal
A6	Tbio Sintonia	Cristalina	Goiás
A7	Tbio Toruk	Giruá	Rio Grande do Sul

Table 2. Rate of emergence, height, incidence and severity of symptoms, mortality, abnormality and transmissibility in seedlings produced from wheat seeds infected with *Pyricularia oryzae* Triticum.

Variable			Samples							Mean	CV (%)
			A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**		
Incidence of PoT[1] in the seeds (%)			8.0	13.5	22.5	32.5	47.0	80.5	0.0	29.2	-
Emergence (%)	DAS[2]	7	74.7 c[3]	85.7 b	95.4 a	85.7 b	85.1 b	54.5 d	97.7 a	82.7	12.0
Seedlings height (cm)	DAS	7	10.4 a	11.4 bc	12.7 b	12.3 b	10.8 bc	3.6 d	15.6 a	11.0	17.0
		14	21.0 b	17.9 c	18.0 c	17.5 c	19.2 bc	15.9 d	24.7 a	19.2	10.0
		21	25.6 c	26.3 bc	28.0 b	27.5 b	31.5 a	20.1 d	31.0 a	27.1	12.0
Symptoms incidence (%)	DAS	7	1.2 cd	0.5 d	1.9 c	4.9 b	5.5 b	10.7 a	0.0 d	3.5	12.0
		14	1.8 de	4.1 d	10.9 c	15.3 b	18.5 b	27.4 a	0.0 f	11.1	9.0
		21	4.2 f	9.7 e	15.3 d	26.3 c	37.3 b	73.4 a	0.0 g	23.7	25.0
Severity (%)	Organ	Root	5.5 cd	7.0 c	11.0 c	18.0 b	25.5 a	31.5 a	0.0 d	13.6	9.5
		Mesocotyl	16.0 c	16.5 c	17.0 c	18.0 c	28.5 b	56.5 a	0.0 d	21.8	10.1
		Primary leaf	6.5 ef	12.0 de	16.0 cd	20.5 bc	25.5 b	91.0 a	0.0 f	24.5	5.6
Mortality (%)	DAS	14	1.5 c	3.0 b	1.5 c	2.8 b	1.7 c	5.4 a	0.0 d	2.8	8.3
		21	19.2 b	15.4 c	13.8 c	20.6 b	13.8 c	48.2 a	4.8 d	20.7	6.1
Abnormal seedlings (%)	DAS	14	0.6 b	2.6 a	1.1 b	1.1 b	2.6 a	2.9 a	0.4 b	1.61	4.4
Transmissibility (%)	DAS	7	15.0 a	3.5 d	8.9 c	15.0 a	11.7 bc	13.3 b	-	9.6	4.5
		14	22.8 d	30.5 c	48.3 a	47.1 a	39.4 b	34.0 bc	-	31.7	10.2
		21	52.9 f	71.7 d	67.8 e	80.8 b	79.3 c	91.2 a	-	63.4	12.1

[1] PoT, *Pyricularia oryzae* Triticum;
[2]DAS, days after sowing;
[3]Means not followed by the same letter, in the line, differ statistically from each other using Tukey's test at 0.05 probability.

Table 3. Correlation analysis between *Pyricularia oryzae* Triticum infection rate in the wheat seeds samples and the variable associated with establishment and sanity of seedlings.

Variable			**Linear correlation coefficient (r)**	**Significance of r (p-Value)[2]**
Emergence (%)	DAS[1]	7	-0.7465	ns
Seedling height (cm)	DAS	7	-0.8530	< 0.05
		14	-0.7207	ns
		21	-0.5627	ns
Symptom incidence (%)	DAS	7	0.9831	< 0.01
		14	0.9793	< 0.01
		21	0.9962	< 0.01
Severity (%)	Organ	Root	0.9731	< 0.01
		Mesocotyl	0.9585	< 0.01
		Primary leaf	0.9446	< 0.01
Mortality (%)	DAS	14	0.8147	<0.05
		21	0.8459	< 0.05
Abnormal plants (%)	DAS	14	0.7295	ns
Transmissibility (%)	DAS	7	0.5276	ns
		14	0.4826	ns
		21	0.7324	ns

[1]DAS, days after sowing;
[2]Level of statistical significance of r according to the critical value in the Student-t distribution; ns, not significant.

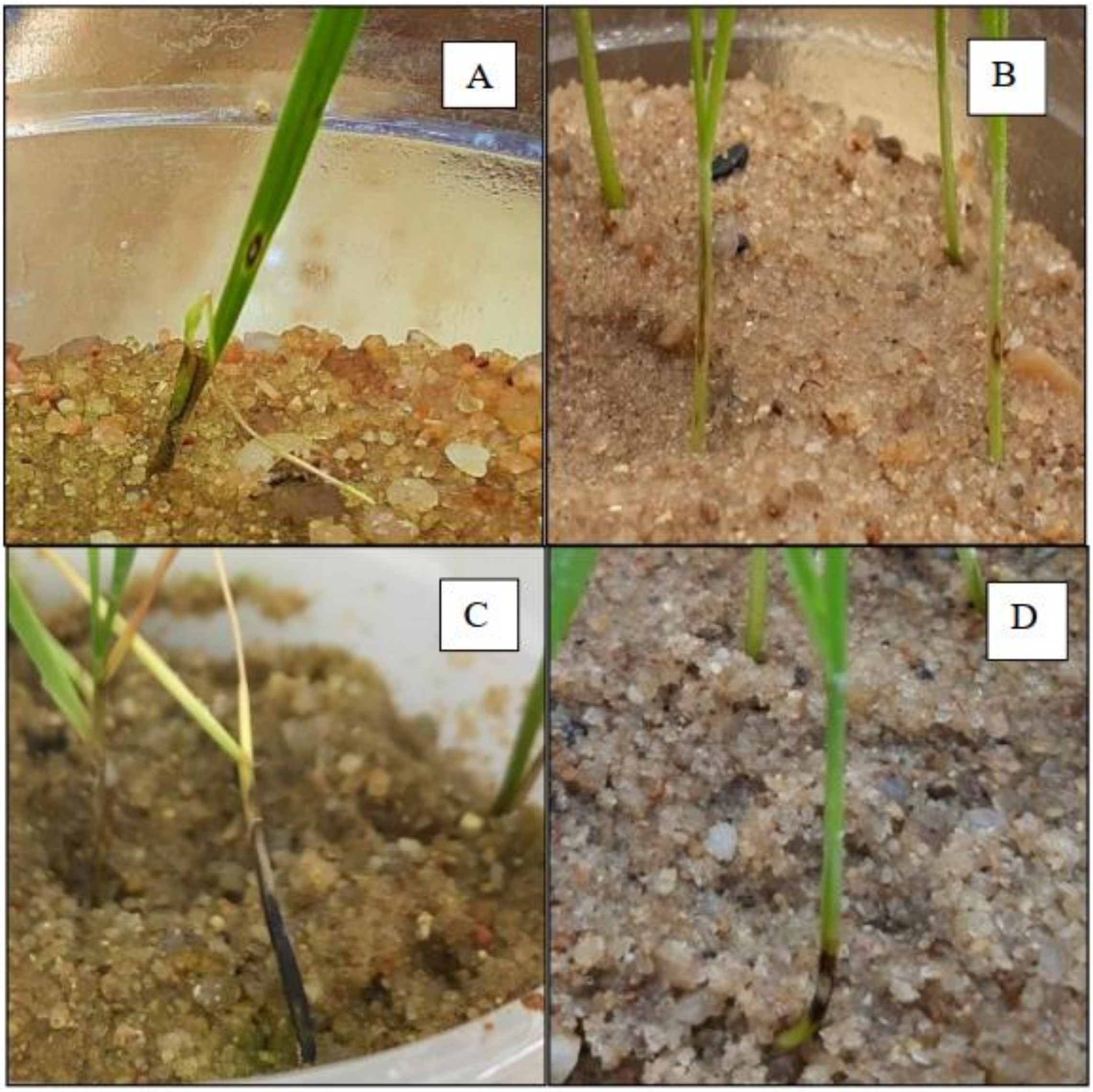

Figure 1. Symptoms observed on wheat seedlings 14 days after sowing: A) elliptical lesion caused by *Pyricularia oryzae* Triticum on the primary leaf of the wheat seedling; B) lesion observed on the stem of a wheat seedling; C) necrosis on the wheat seedling; D) characteristic lesion in wheat seedling.

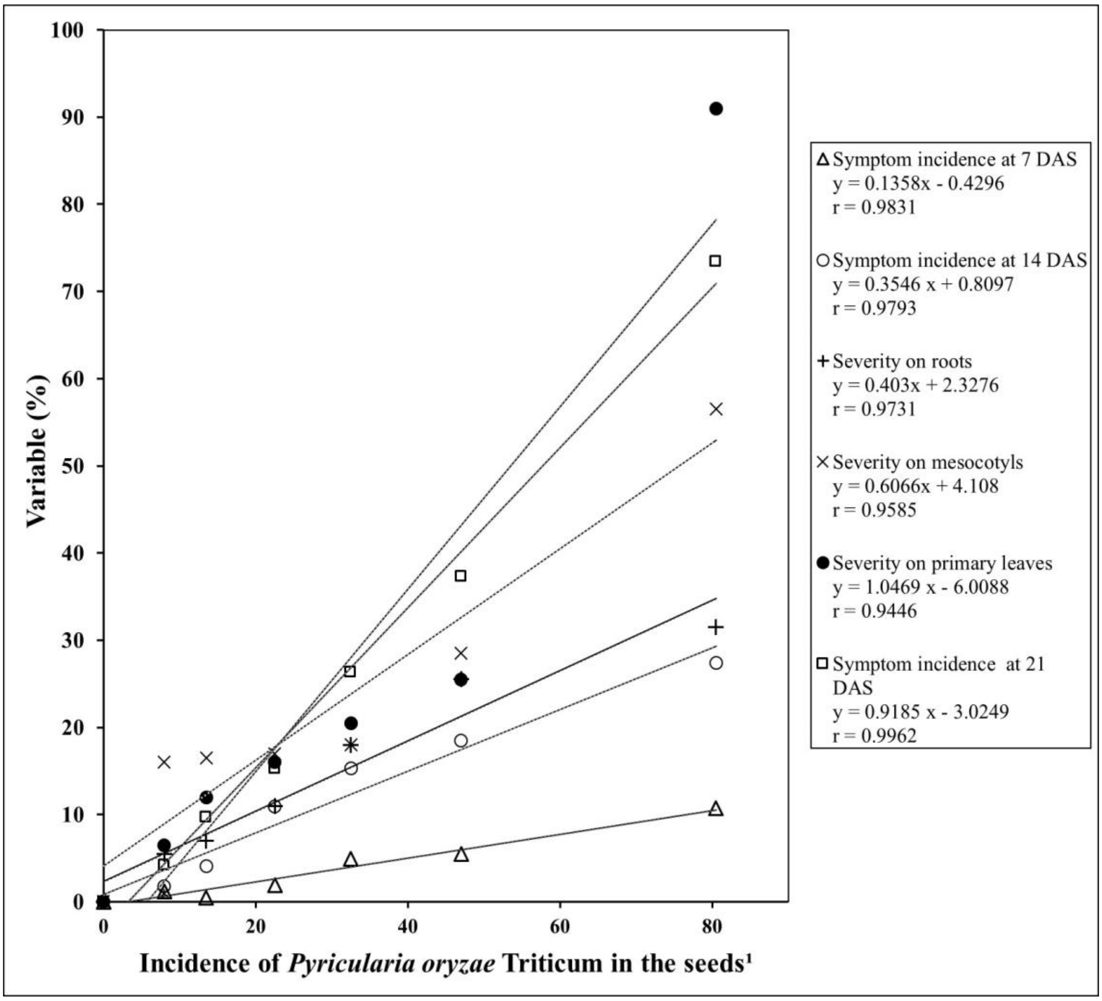

Figure 2. Linear correlation between incidence of *Pyricularia oryzae* Triticum in wheat seeds and incidence and severity of symptoms on seedlings.

[1]All correlations were confirmed at 0.01 probability.
[2]Severity scored at 21 days after sowing (DAS).