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In-vitro Screening for Disease Resistance in Wheat Genotypes against *Bipolaris sorokiniana* Using Callus Culture Method

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was done to identify the efficacy of callus culture method for *in-vitro* screening to identify resistant and susceptible wheat genotypes. The study led to establishment of protocol for *in-vitro* screening of susceptible and resistant wheat genotypes against *Bipolaris sorokiniana*. The *Bipolaris sorokiniana* crude toxin was used at various concentrations to supplement the callusing medium and the response of twelve genotypes was studied. The susceptible genotype Agra local showed maximum area prone to death of the callus because of effect of toxin in medium supplemented with MS+2,4-D (4.0 mgl^{-1}) + NAA (2 mgl^{-1}) with *B. sorokiniana* toxin whereas the resistant genotype Yangmai#6 showed least area affected by the toxin and the cream/ white callus observed in the case of controlled medium supplemented with toxin. The genotypes which were found to be resistant and susceptible in the field condition were clearly identified as the same using this method.

Keywords: Bipolaris sorokiniana; wheat; spot blotch; toxin; callogenesis.

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1. INTRODUCTION

Wheat (*Triticum aestivum*) (2n=28) is a monocot plant. It belongs to the family Poaceae and subfamily Panicoideae and tribe Triticeae. In India, wheat is preferred as one of the most staple food crops in terms of area, production and consumer preference. There are many constraints responsible for low yield of wheat in wheat growing countries. The most important crop wheat suffers from many biotic stresses in the form of disease and pest.

Spot blotch is one of the important seed and soil borne disease caused by the fungus *Bipolaris sorokoniana*. It usually induces symptoms on the leaf, sheath and stem [1]. This pathogen causes various diseases, however, spot blotch of wheat caused by this pathogen is supposed to be one of the most significant diseases in environments which are characterised by high temperatures (coolest month with temperature more than 17°C) and high humidity [2]. The disease spot blotch in wheat caused by fungal pathogens multiply and survive in the rice- wheat cropping system of South Asia and rice harvest act as substrate for *B. sorokiniana* [3]. It is known to cause substantial quantitative and qualitative losses in grain yield.

Bipolaris sorokiniana secrete a toxic compound which is known to be a host specific [4]. Screening for spot blotch disease through natural and conventional methods have constraints because of time requirement, needed much space and season dependent. Toxins play an important role in pathogenicity for the development of diseases in plants which is used to categorize susceptible and resistant wheat genotypes under *in-vitro* conditions [5]. As against this, *in-vitro* selection has emerged as a feasible and cost-effective tool for screening and developing stress resistant plants under controlled conditions with limited time and space. In view of this, the present study has been undertaken to evaluate the use of *Bipolaris sorokiniana* toxin as a tool for assessing resistance of wheat genotypes.

2. MATERIALS AND METHODS

The Potato Dextrose Agar and Potato Dextrose Broth media from *Himedia* were prepared, sterilized and transferred into petri plates and conical flask respectively to encourage exponential fungal growth. The infected leaves were collected from the field of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar in the year 2018-2019. The pathogen of spot blotch disease in wheat i.e., *Bipolaris sorokinina* was isolated and surface sterilized with 0.1% sodium hypochlorite for 1 minute followed by washing thrice with double distilled water and inoculated in petri plates under optimum conditions provided in laminar air flow and incubated at 25±2ºC.

Pathogenicity test was executed on penultimate leaves of 30 days old susceptible wheat cultivar Sonalika by infiltering 100µl of the pure culture filtrate to confirm the ability of the fungus in culture to produce typical symptoms of spot blotch under artificial conditions [6]. *Bipolaris sorokiniana* pathogen culture was purified by regular subculturing on Potato Dextrose Agar petri plates.

The culture filtrate was prepared by inoculating 5mm pieces of fungal mycelium in 250 ml of Erlenmeyer flasks containing 150 ml of PDB medium followed by incubation at 25±2ºC on rotary shaker (150 rpm). The mycelium obtained was filtered through three layers of cheese cloth and Whatman filter paper number 42 by using Buchner funnel under vacuum. For sterilization, the culture filtrate was filter sterilized using Millipore filter 0.22µm. The pure culture filtrate was transparent yellow in appearance. The pH 5.6 was adjusted before use. The filtrate was used for screening at different concentrations of 0%, 1%, 5% and 10% (v/v) [7].

The seeds of selected 12 wheat genotypes PBW-343, Chiriya-3, Sonalika, HD-2967, Agra local, yangmai#6, K-307, UP-2565, HD-3086, HD-2733, Salembo and Cuo/79/Prulla were surface sterilized and pre-treated and left overnight. The mature embryo was excised and placed on culture tubes containing MS + 2,4-D (4.0 mgl^{-1}) + NAA (2 mgl^{-1}) medium. The small pieces of embryogenic callus subjected to callusing medium with varying levels of culture filtrate to achieve concentrations of 1, 5 and 10 µl per ml of growth medium in separate flasks and redistributed equally into three test tubes per treatment of each genotype and incubated at 25±2ºC for 28 days for in-vitro induction and screening of disease resistance. The BCF free medium was also prepared which served as control. After four weeks of inoculation on toxic

medium, callus with considerable growth were selected. The final data was visually observed and scored as white/ cream callus, when it was healthy callus and visualized brown when necrotic areas were observed in the callus tissues [8].

An indefinite disease index (1-9) was established [8] for scoring the effect of culture filtrate in callus. The description of the scale is given below:

1= white/ cream callus, 3= brown necrotic lesions, 5= easily detected brown necrotic callus, 7= mostly area covered with brown necrotic patches and, 9= dark brown necrotic tissue.

The resultant experiments were executed on the basis of three replications based on completely randomized design (CRD) and the observations were subjected to two-way Analysis of variance (ANOVA) to test the significance of the results using the database OPSTAT.

3. RESULTS

The twelve selected genotypes of wheat showed their differential response with respect to callus growth in presence of culture filtrate of *Bipolaris sorokiniana* in the medium as revealed by the conclusion of two-way ANOVA. The effect of toxin treatment at 10 µl/ml of medium was found to be lethal for growth of callus. At this concentration, there was significant reduction in the callus growth (as observed by decrease in callogenesis percentage) on treatment with this concentration of toxin (Table 1). The decrease was observed for all the genotypes. However, the effect of culture filtrate was maximum in the case of Agra local and the least affected genotype was Yangmai#6 followed by Chiriya 3. The growth of callus in presence of culture filtrate of pathogen graded the twelve wheat genotypes for their resistance for spot blotch disease in the following order: Chiriya-3, yangmai#6, Cuo/79/Prulla, HD-3086, HD-2967, Salembo, K-307, HD-2733, PBW-343, Sonalika , UP-2565 and Agra local.

4. DISCUSSION

For the twelve wheat genotypes experimented *in-vitro*, the vulnerability to the toxin amplified with the increase in concentration of the toxin. The extent of damage to the callus tissue was directly linked with the dose [9]. This may be attributed to the increased intensity of metabolic processes such as increased heat emission to counter fungal metabolites [10]. 10µl/ ml medium concentration was found to be the lethal dose for the genotypes used in the present study. At this concentration, all the genotypes showed significant reduction in callogenesis percentage. The reduction may be due to the detrimental effect of the toxin on inducing cell death in the callus tissues which prevent their growth [11]. Consequently, the necrosis of the callus tissues was observed at this concentration for all the genotypes.

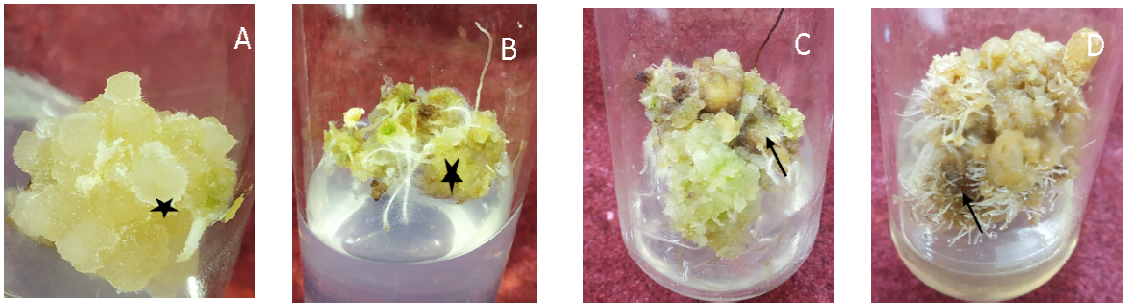

Fig. 1. Effect of toxin on callogenesis of wheat genotypes. [a. control calli of wheat genotype Agra local, b. calli treated with 1% B. sorokiniana culture filtrate, c. calli treated with 5% B. sorokiniana toxin and d. treated callus at lethal dose (10%). *indicates cream callus and ↗ indicates dark brown necrosis]

Table 1. Effect of culture filtrate on callogenesis of twelve wheat genotypes

	PBW-343	Chiriya-3	Sonalika	HD-2967	Agra local	yangmai#6	K-307	UP-2565	HD-3086	HD-2733	Salembo	Cuo/79/Prulla
Control (µl/ml)	98.34±1.67[1]	88.33±1.67[1]	91.66±1.67[1]	91.66±1.67[1]	86.66±1.67[1]	101.96±1.67[1]	96.66±1.67[1]	83.33±1.67[1]	91.66±1.67[1]	88.33±1.67[1]	91.66±1.67[1]	98.33±1.67[1]
1	86.67±1.67[3]	88.33±1.67[1]	81.66±1.67[3]	88.33±1.85[3]	61.66+1.67[5]	82.22+1.67[1]	86.66+1.67[3]	71.66+1.67[3]	86.66±1.67[3]	78.33+1.67[3]	81.66+1.67[3]	88.33+1.67[3]
5	61.67±1.67[5]	78.33±1.67[3]	58.33±1.67[7]	78.33±1.85[5]	53.33+1.67[7]	76.19+1.67[3]	73.33+1.67[5]	61.66+1.67[5]	73.33±1.67[5]	68.33+1.67[5]	68.33+1.67[5]	73.33+1.67[5]
10	36.67±1.67[9]	68.33±1.67[5]	31.66±1.67[9]	48.33±1.76[7]	38.33+1.67[9]	76.66+1.67[3]	48.33+1.67[7]	36.66+1.67[9]	58.33±1.67[7]	51.66+1.67[7]	63.33+1.67[5]	51.66+1.67[7]
Mean B	70.833	80.83	65.83	76.66	60.00	84.26	76.25	63.33	77.50	71.667	76.25	77.91
Factors	**C.D.**	**SE(d)**	**SE(m)**									
Factor (A)	1.424	0.716	0.507									
Factor (B)	2.467	1.241	0.877									
Factor (A X B)	4.934	2.482	1.755									

The necrosis was more in the genotype Yangmai#6 and least in the genotype Agra local. It was observed that the resistant genotypes showed less necrosis and less decrease in callogenesis percentage on the medium containing the lethal dose as compared to susceptible genotypes. Yangmai#6 is the resistant genotype and Agra local is the susceptible genotype based on the field evaluation experiments [12]. Hence, the response observed by Agra local is the least. The decrease in callogenesis percentage and increased necrosis in susceptible genotypes may be due to enhanced ethylene production in response to *B. sorokiniana* toxin. Hodges and Campbell [13] reported that the susceptible genotypes of *Poa pratensis* showed enhanced ethylene production which causes damaged to chloroplast leading to necrosis. However, the resistant genotypes showed cellular defence responses like CWA formation, decreased mesophyll invasion and decreased spreading within the mesophyll which help to counteract the toxin induced necrosis [14].

Thus, the present study helps to establish that the *in-vitro* screening can be used to identify susceptible and resistant wheat genotypes based on their response to lethal dose of B*. sorokiniana* crude toxin.

5. CONCLUSION

The present investigation was done to identify the efficacy of callus culture method for in-vitro screening to identify resistant and susceptible wheat genotypes. The results of the experiments were able to differentiate between susceptible and resistant genotypes. Hence, the study put forward an efficient and easy method for in-vitro screening to identify wheat genotypes resistant and susceptible against *Bipolaris sorokiniana*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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