### Morphological Signs of Barley Spot Pathogens in the Conditions of the Almaty Region

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Corresponding Author: Nadira Zhumakhanovna Sultanova Kazakh Research Institute for Plant Protection and Quarantine, Almaty, Kazakhstan Email: nadira.sultanova@mail.ru Abstract: The most widespread barley diseases in the southeastern region of Kazakhstan are common root rot, dark brown spots (caused by Bipolaris sorokiniana Shoem) and stripe disease (Drechslera graminea). This study presents the results of the morphological and cultural characteristics of fungi causing these diseases. The occurrence of the B. sorokiniana, fungi on barley leaves averaged 6.5%. They are more often was found with fungi from the genus Alternaria (35.5%). The D. graminea and Alternaria were registered in 25% of the samples. The optimal nutrient media for the cultivation of B. sorokiniana, were namely V4 medium, potato agar and Czapek's medium. The optimal temperature for the growth of colonies of *B. sorokiniana* were ranged from 22 to 30°C. D. graminea conidia isolated from natural substrates had a statistically significant increase in length, width and the number of septa compared to conidia grown on an artificial V4 nutrient medium. The germination energy of barley equaled 71-73% for the Astana 2000, Preriva and Symbat barley varieties, 79% for the Sever variety, 88% for the Arn variety and 96-96.5% for the Donetskiy 8 and Ilek 9 varieties.

Keywords: Barley, Seeds, Bipolaris sorokiniana, Drechslera Graminea

### Introduction

Spring barley is an important food crop in Kazakhstan; it is cultivated on an area of 1,881 thousand ha with an average yield of 13.1 c/ha (FAO, IFAD, UNICEF, WFP and WHO, 2018; GRK, 2018). Usually, it is placed in a crop rotation with the 3rd or 4th crop after fallow ground. The yield of barley is influenced by diseases with soil infections, which include common root rot (caused by Bipolaris sorokiniana Shoem., Fusarium spp.) and dark brown leaf spot (Bipolaris sorokiniana Saccin Sorok) Shoem (Agrios, 2005). Symptoms of diseases appear on barley in the form of leaf spots and root rot on seedlings, ears, stems, leaves, parts of flowers. The yield of barley grain, in this case, decreases by 10-50%. The most widespread barley diseases in the South-Eastern region of Kazakhstan are common root rot and dark brown spots (caused by B. sorokiniana), stripe disease (Drechslera graminea), net blotch (D. teres) and bordered leaf blotch (Rhynchosporium secalis) (Bekezhanova, 2009). Successful control of these diseases depends on the use of resistant varieties, seeds that are free of pathogens, seed treatment with fungicides, crop rotation and fertilization, plowing of contaminated crop residues and application of fungicides (Agrios, 2005; Lucas, 2020; Sagitov *et al.*, 2020). Besides, phytopathogenic fungi lead to changes in various chlorophyll fractions in diseased plants (Kuldubayev *et al.*, 2020; Dutbayev *et al.*, 2020a-b).

Recent studies on the study of barley diseases with soil infection were carried out to develop a strategy for protecting barley seedlings from infection caused by Sclerotium rolfsii using soil infested with the antagonist fungi Trichoderma (Faruk, 2019), study the effect hydrothermal stress on the development of soil infections in wheat and barley (Toropova et al., 2018), evaluate the effectiveness of pre-sowing seed treatment against infection caused by Fusarium spp. and Cochliobolus sativus (Sooväli et al., 2017), develop protective measures (Gangwar et al., 2018; Høyer et al., 2019; Singh, 2017), identify the soil-habitat barley mosaic virus (Cadot et al., 2017) and study the antagonistic activity of bacterial species of the Bacillus genus on the B. sorokiniana barley as pathogenic fungi (Harba et al., 2020). Currently, there is no research in Kazakhstan on barley diseases with soil infection.



© 2021 Nadira Zhumakhanovna Sultanova, Alibek Maratovich Uspanov, Madina Malikovna Bekezhanova, Alua Manatbekkyzy Rysbekova and Gaziza Bazarbaevna Sarsenbayeva. This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license. The purpose of our research was to study the morphological and cultural characteristics of fungi causing barley leaf spot disease, *B. sorokiniana* and *D. graminea*, in the South-East of Kazakhstan. During the study, we found that the occurrence of the *B. sorokiniana* on barley leaves in the foothills of the Almaty region averaged 6.5%, together with fungi from the genus *Alternaria* (35.5%). *D. graminea* + *Alternaria* were found about 25% of the samples. We determined the optimal nutrient media for the cultivation of *B. sorokiniana* and *D. graminea*. We also established the morphological characteristics of conidia and their colonies.

### Methods

When studying the biological characteristics of pathogens of dark brown and striped spots, barley was grown in soil, plastic vessels, humid chambers and artificial agar nutrient media. Barley seeds were sown in wet sand or soil filled with plastic containers to determine the extent of pathogen infestation. For these purposes, seeds were sown 3-4 cm deep in plastic cups, were kept in a laboratory in front of a window or a special flora at 15°C for 14-20 days (Agrios, 2005).

To isolate a pure culture of the *B. sorokiniana*, the fungi mycelium and conidia formed in leaf fragments in a humid environment were inoculated onto V4 artificial media, Czapek's medium, potato agar, oat agar containing vegetable juices and agar. They were kept in a thermostat at 22°C for 7-10 days.

Then, pure cultures of the pathogen were isolated, which were cultured on V4 nutrient culture medium and incubated for 8 days at 23°C for 12 h in the light and 12 h in the dark (Agrios, 2005; Barbedo, 2019). Composition of V4 medium from vegetable crops: V4 Consisted of 150 mL of vegetable juice from the following crops: 60 mL of beetroot juice, 45 mL of celery juice, 30 mL of carrot juice, 15 mL of tomato juice, 15 g of agar, 3 g of CaCO<sub>3</sub> and 850 mL of distilled water.

To determine the optimal temperature for the growth of colonies of *B. sorokiniana* isolated from pieces of barley leaves from the Almaty region, it was grown in pure V4 culture medium in petri dishes at different temperatures: 5, 13-15, 16-18, 22, 30 and 41°C in four replicates. Then, on days 5, 8, 10 and 15, the growth rate of fungal colonies was determined.

The paper roll method was used to determine the ability of transmission of pathogens of *Helminthosporium* spots through seeds. For this, 40 seeds were sown on two layers of sterile blotting paper ( $15 \times 105$  cm) at a distance of 2 cm. Then the paper was moistened with sterile water and covered with a second sheet of moistened paper ( $5 \times 105$  cm). The bags were placed in glass or plastic containers filled with an equal amount of water and kept in a thermostat at 20°C for 3-4 days. Then they were

grown for 10-12 days in front of a window or an installation with special lamps. After 12-14 days, the sprouted seeds and seedlings were carefully examined for the presence of leaf spots and root rot. To determine the biometric characteristics of fungi species, the length and width of 100 conidia and the number of septa were measured (Agrios, 2005; Barbedo, 2019).

To determine the effect of different temperatures on the growth of the colonies of the *D. graminea*, we grew its culture in thermostats at 10, 16, 20, 25, 29 and 37°C on the V4 artificial medium. The diameter of the fungi colonies was examined after 15 days.

The following method was used to determine the presence of *Bipolaris* and *Drechslera* in the seeds of barley varieties in the southern and southeastern regions: 25 Grains were grown in four replicates in a humid chamber at 25°C. After seven days, they were examined and if a plaque was observed on the surface of the grain, it was cut on glass slides and the species composition of the fungi was determined under a microscope. To determine the optimal temperature required for the growth of pure cultures of the *B. sorokiniana* and *D. graminea*, they were cultured in four replicates of V4 nutrient medium in petri dishes at 3-5, 13-15, 16-18, 22, 30 and 41°C. Colony diameters were measured after 5, 8, 10 and 15 days (Agrios, 2005; Barbedo, 2019).

Barley seeds received from Agropark Ontustik LLP were assessed on the 3rd and 5th days and the laboratory germination was assessed on the 7th day. The sowing quality of seeds was determined in humid chambers placed in a thermostat at a temperature of 24°C. The germination energy was taken into account on the 3rd day after the beginning of the experiment and the laboratory germination was evaluated on the 7th day, according to the number of germinated seeds (Barbedo, 2019).

The analysis of variance was performed using the R software. The acceptable level of significance was determined using the P-value (Aphalo, 2017).

### Results

We studied the morphological and cultural characteristics phytopathogenic fungi B. sorokiniana and D. graminea caused barley leaf spots in the South-East of Kazakhstan. A pure culture was isolated, the pathogenicity of the strains was determined and the spotting pathogens were identified. During our research, we paid special attention to the biological characteristics of pathogens, methods of preservation, the spread of Helminthosporium diseases in barley, the growth and development of pathogens. This is necessary for the correct diagnosis of phytopathogens and the choice of effective ways to carry out protective measures against diseases. To do this, it is necessary to understand the morphological characteristics biological and of pathogens. In some cases, they may vary depending on the growing medium and growing conditions of the plant, crop varieties and affected organs attacked by the dark brown spot disease (*B. sorokiniana*). The occurrence of the *B. sorokiniana* on the leaves of barley growing in the foothills of the Almaty region was estimated; the average occurrence equaled 6.5%. The phytopathogenic fungi was often found together with *Alternaria* (35.5%). The presence of *D. graminea* + *Alternaria* was found in 25% of the samples (Table 1).

After 3-4th days, the fungi colonies began to appear on the nutrient media. The colonies were initially pale; as they grew, their color gradually became olive, dark green, rising above the substrate, thickening and becoming denser. Within 10-15th days of colonies at the optimal temperature and optimal nutrient medium, the fungi colonies spread over the entire area of the petri dishes and due to the formation of a large number of spores, the fungi colonies had a velvety, dark green, or olive color. The conidia were brown or yellow, elliptical, upright, or slightly curved. The length range conidia of the *B. sorokiniana* was 18-77, 47.4  $\mu$ m at the average, the width ranged from 11-22, 16.6  $\mu$ m at the average (Table 2).

## Effect of Different Temperatures on the Growth of the B. Sorokiniana

The development of plant infectious diseases is directly related to environmental conditions, especially temperature and humidity. These factors directly affect the growth of pathogenic spores and conidia, the penetration of their tubules into plant cells, as well as the spread and development of diseases. Therefore, it is important to know the optimal temperature for the growth of pathogens and its low and high values (Agrios, 2005).

During the experiment, it was found that the optimal temperature for the growth of fungi colonies ranged from 22 to 30°C. It was found that different temperatures significantly affected the growth rate of B. sorokiniana colonies, as well as the morphological features of their development. At low temperatures (5°C) the colonies were light gray and at high temperatures (22°C) they became dense, velvety, dark brown-olive, or dark brown. At a temperature of 16-18°C, the center of the colonies became denser, gray and the edges were pale olive, with clearly visible circles. At 22°C, the edges of the colonies resembled the crown of carnation petals. The fungi grew very quickly at 30°C; therefore, in 7-8 days it covered the entire petri dish and the airy fibers looked like gray wool. To determine the sowing characteristics and variability of the B. sorokiniana substrate isolated from barley, the phytopathogenic fungi isolate from the Almaty region was grown on V4 agar medium, Czapek's medium and nutrient media containing boiled potatoes and oats at a temperature of 25°C. After 15-20 days, the sowing properties and diameter of the fungi growing on various nutrient media, as well as the biometric parameters of its conidia, were determined (Fig. 1).

There was a rapid growth of colonies of phytopathogenic fungi *B. sorokiniana* on a V4 nutrient medium prepared based on several vegetable juices and artificial nutrient media containing potatoes. The fungi also grew well on Czapek's nutrient medium, where the mycelium filaments were very dense and the edges were uneven. However, the growth of the fungi on oat agar was slow and the mycelium filaments were very thin. As for the biometric parameters of fungal conidia, their length and width were significantly higher in the V4 culture compared to other media (Table 3 and Fig. 2).

### Barley Stripe Disease (D. graminea)

Barley leaves with stripe disease were collected at KazNIIZiKR in 2020. First, we examined them under a microscope to find fungi spores. When moistened, a plaque was formed from conidia and conidiophores of the *D. graminea*. The conidia had a cylindrical shape, slightly rounded at the ends, colorless, with light yellow or light brown scales (Fig. 4). Micrometric measurements showed that the length of the conidia ranged from 46 to 150  $\mu$ m and the width from 11 to 25  $\mu$ m, with the number of septa from 1 to 5.

In dry weather, spore-forming organs of the pathogen did not form on leaves with stripe disease. When plant samples were kept in petri dishes in a humid environment for about a week, the size of the fungi was observed to be 0.3 mm. Taking into account that the biometric parameters of microorganisms change depending on the nutrient medium, conidia of the *D. graminea* were isolated from leaves in a humid medium on petri dishes and seeds using the paper roll method (Fig. 3). Further, a pure culture of the fungus was cultivated on a V4 nutrient medium.

It was found that conidia of the phytopathogenic fungi *D. graminea* isolated directly from natural substrates (leaves and grains) had a statistically significant greater length, width and the number of septa in comparison with conidia grown on the V4 artificial nutrient medium (Table 4).

# *Physiological and Biological Characteristics of the D. graminea*

At a temperature of  $10^{\circ}$ C, the colonies grew slowly and after 15 days the diameter of the plant barely reached 40 mm. The structure of the colony was uneven. It was established that the optimum temperature for the growth of fungi was in the range of  $20-30^{\circ}$ C. On day 10th, the fungi colonies were completely covered in the petri dish. Morphological and seeding differences were observed in the growth of the fungi at different temperatures. On day 10th, when cultivating *D. graminea* at  $10^{\circ}$ C, the air mycelium of the fungi was whitish, purple and slightly elevated. The structure of the colony was even. The substrate mycelium was pale, the circle barely visible. The sexual reproduction organs (fruiting bodies) were not formed. When cultivating fungi at 20°C on the 10th day, the air mycelium of the fungi was curly, light gray. The substrate mycelium was light gray, olive in color, without rounding. The sexual reproduction organs of fungi (fruiting bodies) were formed. When cultivating fungi at 25°C on the 10th day, the air mycelium of the fungi was loose, of light gray, olive color. The substrate mycelium was light gray, olive and brown in the middle. The sexual reproduction organs of fungi (fruiting bodies) were formed (Table 5).

Barley seeds from the Almaty region were infested with *B. sorokiniana*, *D. teres* and *D. graminea*. The seed population was affected by both variety and infection factors. Most of the seeds were populated by the *B. sorokiniana* and *D. graminea* fungi. The Donetskiy 8 variety was the least populated with phytopathogenic infection (27%), while other varieties were infested with it by 60-84%.

The most diseased seeds were infested with *B. sorokiniana*. As a result of the analysis of variance, it was found that on days 3 and 5, the average germination energy in the groups was not equal (P-value = 0.01). In the Astana 2000, Preriya and Symbat varieties on day 7, it equaled 71-73%, in the Severny variety, it was 79%, in the

Arna variety, it amounted to 88% and in the Donetskiy 8 and Ilek 9 varieties, it was at the level of 96-96.5%.

According to the laboratory germination parameter (P-value = 0.03), the average values in the groups were also not equal. In Astana 2000, it was 92%, in Symbat and Arna it amounted to 93-94%, in Symbat and Arna it equaled 93-94%, in Preriya, Donetskiy 8, Severny 1 varieties it was 96-96.5% and Ilek 9 had the maximum level of 100% (Table 6).

The seed population was affected by both variety and infection factors. In the Donetskiy 8 variety, the proportion of diseased seeds was 27%, in Sever 1 and Ilek 9, it equaled 59-63%, in Astana 2000 and Preriya, it amounted to 68-74% and in Symbat and Arna, it was 82-84%.

**Table 1:** Isolation of pathogens of *Helminthosporium* diseasesfrom barley samples (Kazakh Scientific ResearchInstitute of agriculture and Crop Production(KazNIIZiR) Zhalpaksay, 2020

	Number of
Fungi	samples, (%)
Alternaria+ B. sorokiniana	35.0
B. sorokiniana	6.5
D. graminea+ Alternaria	25.0
P-value at 99% confidence interval	< 0.01

Table 2: Biometric parameters conidia of the B. sorokiniana isolated from barley plants KazNIIZiR, Zhalpaksay, 2020

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Statistical indicators	Length, µm	Width, µm	Number of partitions, pieces		
Average	99.7	15.6	6.1		
Median	111.0	16.0	6.0		
1st quartile	87.3	12.0	5.0		
3rd quartile	118.0	18.2	7.2		
Minimum	39.0	5.0	2.0		
Maximum	125.0	13.0	13.0		

 Table 3: Growth of colonies of the B. sorokiniana on various artificial nutrient media Kazakh Research Institute of Plant Protection and Quarantine, 2019

	Colony diameter	Conidium		Morphological features of the fungi			
Nutrient medium	(mm)	Length (µm)	Width (µm)	Aerial mycelium	Substrate		
$V_4$	850	850	18	Grows quickly, the surface is loose, velvety, dark brown, with smooth edges	Black color, no radial lines and circles are observed		
Czapek's nutrient medium	850	710	15	Grows well, shades of gray or dark olive or green, uneven edges	Gray, gray-black, no radial lines or circles		
Potato agar	850	730	17	Grows intensively, the surface is velvety, dark olive green, with uneven edges	gray-black, no radial lines or circles		
Oat agar	850	710	16	Grows poorly, cobweb mycelium, light gray, smooth edges	Radiality and zoning are pronounced		
P-value at 95%	0.01	0.03			-		

Table 4: Biometric indicators of D.	g <i>raminea conidia</i> Kazakh R	esearch Institute of Plant Protect	ction and Quarantine, 2019	
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	Lengui (µiii)		widui (µiii)		Number of septa	
Conidia isolated from	Mean range	Average value	Mean range	Average value	Mean range	Average value
Leaves	37-106	74.30	11-25	17.40	1-5	3.60
Grains	46-116	82.70	16-21	17.30	1-5	3.40
V <sub>4</sub> nutrient medium	32-85	66.7 0	14-18	16.00	1-4	2.60
P-value at 95% confidence interval		0.04		0.02		0.01

 Table 5: Sowing and morphological characteristics of the D. graminea at different temperatures Kazakh Research Institute of Plant

 Protection and Quarantine, 2019

	Temperature (°C) and description	Temperature (°C) and description					
Parameters	10	20	25				
Organ	uneven	even	even				
Aerial mycelium Substrate mycelium	Whitish, purple, slightly raised Pale, barely visible circle	Wavy, light grey Light gray, olive color,	Loose, light gray, olive color				
Fruiting body	not formed	without rounding formed	Light grey olive, brown in the middle formed				

Table 6: Results of laboratory seed germination and the intensity of settlement by *Helminthosporium* Kazakh Research Institute of Plant Protection and Quarantine, 2020

	The energy of germination on. (%)		Laboratory		The population of seeds		
			germination	Percentage of			
Variety	Day 3	Day 5	on day 7 (%)	infested seeds (%)	B. sorokiniana	D. teres	D. graminea
Astana 2000	34.0	71.0	92.00	68.0	+++	++	+
Preriya	35.0	73.0	96.00	74.0	+++	++	+
Donetskiy 8	57.0	94.8	96.50	27.0	+++	++	+
Ilek 9	51.0	95.0	100.00	59.0	+++	+	+
Symbat	32.5	72.0	93.00	82.0	++	+	+
Arna	46.0	88.3	94.00	84.0	++	+	+
Sever 1	32.0	79.0	96.00	63.0	++	+	+
P value at 95% confidence interval	0.01	0.01	0.03	< 0.01			



Fig. 1: Growth of a *B. sorokiniana* colony at different temperatures

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Fig. 2: B. sorokiniana conidium Kazakh Research Institute of Plant Protection and Quarantine, 2019



Fig. 3: The paper roll method for evaluating seed germination energy of barley Kazakh Research Institute of Plant Protection and Quarantine, 2019



Fig. 4: D. graminea conidium Kazakh Research Institute of Plant Protection and Quarantine, 2019

### Discussion

According to (Bekezhanova, 2009), the most widespread barley diseases in the southeastern region of Kazakhstan are common root rot and dark brown spot (caused by *B. sorokiniana*), stripe disease (*D. graminea*), net blotch (*D. teres*) and bordered leaf blotch (*R. secalis*). She found that strains of the phytopathogenic fungi *B. sorokiniana* isolated from leaves of barley, rye and wheatgrass differed in morphological and cultural characteristics and pathogenic properties. *B. sorokiniana* can infect rye, barley and wheatgrass, possibly transferring them from rye to barley and wheatgrass and, accordingly, from wheatgrass to these crops.

On barley, the fungus *Bipolaris sorokiniana* can be found jointly with fungi from the genera *Alternaria* sp., *Fusarium* sp. and *Penicillium* sp. (Puchkova *et al.*, 2020). According to our data, the occurrence of *B. sorokiniana* isolates on barley leaves in the foothills of the Almaty region was 6.5%. It was found together with fungi from the genus *Alternaria* (35.5%) and *Drechslera graminea* + *Alternaria* (25%).

According to (Kumar and Rai, 2018), out of nine growth media (potato dextrose, Asthana Hawker's, Czapek's, Richards', malt extract, oatmeal, standard nutrients, cornmeal and wheat leaf decoction media) tested, oatmeal medium was the best-suited medium for radial growth and biomass production of *Bipolaris sorokiniana* as highest colony diameter, as well as dry mycelial weight was recorded on this media. For sporulation of *Bipolaris sorokiniana*, potato dextrose agar media was best as the highest numbers of spores/ml were recorded in this media. According to our data, the optimal nutrient media for the cultivation of *B. sorokiniana* were determined-V4 medium, potato agar and Czapek's medium. The optimum temperature for the growth of fungal colonies was 22-30°C.

Turuspekov *et al.* (2016) reported that genotyped a collection of barley of 92 commercial varieties and prospective lines, where resistance to stem rust was found in the field against an artificial infectious background of the Research Institute of Biological Safety Problems in the Zhambyl region of Kazakhstan. GLM analysis identified 15 SNPs associated with seeding time resistance (HA) and two SNPs associated with milky-wax seed maturity.

Rsaliyev *et al.* (2017) evaluated 107 isolates of the pathogen of powdery mildew from seven populations found on cultivated barley in two geographical points of Kazakhstan in 2015 and 2016. All isolates were virulent for the Mla8 resistance gene and avirulent for the Mla9, Mla 1 + Mla 2, Mla 6 + Mla14, Mla13 + MlRu3, Mla7 + MlNo3, Mla10 + MlDu2, Mla13 + MlRu3 and Mlo-5 resistance genes. There were 23 pathotypes with virulence complexity ranging from 1 to 6. In all populations of the South Kazakhstan and Zhambyl regions, pathotypes 24 and 64 predominated.

Yield losses on cereal crops from leaf spots can increase depending on the cultivar's resistance to disease and weather conditions (Tamang *et al.*, 2020; Surin *et al.*, 2021). According to our data, in the Donetskiy 8 variety, the proportion of diseased seeds was 27%, in Sever 1 and Ilek 9, it equaled 59-63%, in Astana 2000 and Preriya, it amounted to 68-74% and in Symbat and Arna, it was 82-84%.

### Conclusion

We studied the morphological and cultural characteristics of fungi causing barley leaf spots, *B. sorokiniana* and *D. graminea*, in the South-East of Kazakhstan. The occurrence phytopathogenic fungi *B. sorokiniana* on barley leaves in the foothills of the Almaty region averaged 6.5%. The fungi occurred more often with fungi from the *Alternaria* (35.5%). *D. graminea* + *Alternaria* were found in 25% of the samples. We determined the optimal nutrient media for the cultivation of *B. sorokiniana*, namely the V4 medium, potato agar and Czapek's medium. The optimum temperature for the growth of fungi colonies equaled 22-30°C.

*D. graminea* conidia isolated directly from leaves and grains had a statistically significant greater length, width and the number of septa compared to conidia grown on an artificial V4 nutrient medium. We described the morphological and cultural features of colonies of *B. sorokiniana* and *D. graminea* fungi on artificial nutrient media. The indicators of germination energy on days 3 and 5 differed statistically significantly: In the varieties Astana 2000, Preriya and Symbat, it was 71-73% on day 5, in Sever, it equaled 79%, in Arn, it was 88% and in the varieties Donetskiy 8 and Ilek 9, it was at the level of 96-96.5%.

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### **Author's Contributions**

All authors equally contributed in this work.

### Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved

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