

Macroanalysis of Winter Wheat Seeds and Features of their Germination

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ABSTRACT

The peculiarities of the primary visual inspection of winter wheat seeds are considered. In the North-East of Ukraine, three groups of abnormal seeds (wrinkled, small seeds, and with a black point) were being identified by macroanalysis during 2017–2022. Factors affecting the representation of these groups in the samples were analyzed. It was determined that the amount of abnormal seeds is determined by the growing conditions of wheat, the place of cultivation and spraying of wheat with fungicides. The maximum number of substandard seeds was noted in Polissia, where black point dominated. In the Forest-steppe, the largest amount of such seeds was recorded in 2022, among which small seeds prevailed. A study of the characteristics of the manifestation of a black point showed that the blackening of the embryo occurs only from above. A seed with a black point is usually mature. For the first time, it was proven that spraying plants with essential oils of cinnamon and fennel leads to an increase in the appearance of the black point, which once again confirms its abiotic nature. The study of the germination of externally healthy and abnormal seeds of the Bohdana variety of the 2018 and 2019 harvests under conditions of low temperature and over moistening showed better indicators for the first group. But the germination of abnormal seeds had contradictory results, especially in different years. Comparison of the germination and development of seedlings from healthy and blackening seeds under favorable conditions during different periods of storage of wheat samples, from different places of cultivation, on different genotypes gave new unexpected results: higher indicators in the variant with black point. During further study in conditions of low temperature, which is explained by very late sowing of winter wheat in recent years in Ukraine, completely different data were obtained. Seed germination was better in healthy seeds. Seedlings developed faster at first also from healthy seeds, but on the last two dates of measurement, they began to grow faster from seeds with black point.

Keywords: winter wheat, small and wrinkled seeds, black point, seed germination, seedling development.

INTRODUCTION

Wheat in Ukraine ranks third among other crops in terms of cultivated area, after corn and sunflower. Annually, this culture is sown on approximately 6.5 million hectares. In recent years, the yield of winter wheat has varied between 3.8 and 4.6 tons per ha. Sumy region has insignificant cultivated areas, compared to other regions of Ukraine (on average for 2019–2020,

it was 179.9 ha), but occupies the highest ranks in terms of crop yield. Thus, in 2020, agrarians of our region of various forms of ownership received the maximum figure in the country – 5.4 tons per hectare. Despite the aggression from Russia, the temporary occupation of the Sumy region, this year's harvest was also high. Visual diagnostics helps to identify abnormal seeds of agricultural crops at the first stages. For example, a special guide (2015–2016) was developed to

identify defective seeds in Australia. In Ukraine, for wheat seeds, an impurity (small) is detected; for grain, in order to establish the appropriate class, it is necessary to determine the sporulation by bunt fungi, the percentage of fusarium-infected and grain with a black point. The last two indicators are determined visually. Thus, grains with *Fusarium* sp. are radically different from healthy ones in terms of external features. Fusarium-infected grains are described as thin, wrinkled with a depressed deep groove and pointed barrels, discolored surface, brittle endosperm, presence of mycelium and sporulation in the groove, non-viable embryo (Gagkaeva *et al.*, 2011; Birr *et al.*, 2022). *Fusarium graminearum* sensu stricto [rarely *F. culmorum* (W. G. Smith) Sacc.] causes similar symptoms in natural conditions. But the phenomenon of hidden infection is known for *Fusarium* sp. Therefore, the percentage of infection when diagnosed by special methods (biological, molecular) is often much higher than the presence of typical fusarium-infected seeds. The manifestation of the infection depends on the type of fungus, its aggressiveness, conditions of infection and the course of the infectious process. Such species as *F. sporotrichioides* Sherb. and *F. poae* (Peck) Wollenw. often do not cause external signs of grain infection (Gagkaeva *et al.*, 2011). The harmfulness of Fusarium head blight consists in reducing the quantity and quality of the crop. A proven decrease in the weight of 1000 seeds, grain quality indicators (Chami *et al.*, 2022) and dough (Spanic *et al.*, 2021) The most dangerous for humans and animals is the accumulation of mycotoxins (Ferrigo *et al.*, 2016). Black point on seeds was previously associated with the presence of bacteria and fungi of the following genera: *Alternaria* sp., *Cochliobolus* sp., *Fusarium* sp., *Cladosporium* sp., *Curvularia* sp., *Penicillium* sp., *Aspergillus* sp., *Stemphylium* sp. etc. But the research of the last decades explained the blackening of the embryo with biochemical reactions in the grain: the high activity of peroxidases that oxidize phenolic substances (Hannibal, 2014). It was proved that the extracts from the blackened parts of the grain differed from the melanin of *Bipolaris sorokiniana* (Sacc.) Shoemaker (Li *et al.*, 2020). Some researchers have ruled out biotic factors as the cause of the black germ, pointing to the connection of symptoms with the formation of stress and other proteins (Hannibal, 2014). Black point in Ukraine does

not have a particularly negative effect on seeds germination. At the same time, in other countries with high humidity, this symptom is associated with non-germination of seeds, its rotting and necrosis on the leaves of seedlings and inhibition of their development (Rani and Sharma, 2018; Sharma *et al.*, 2021). In our country, the price of grain products depends on the amount of colored grain.

The primary macroanalysis of wheat is very important in Ukraine, the quality of the grain depends on it, that is, its price on the market of agricultural products. And the issue of the germination of individual groups of abnormal seeds (especially with black point) has not been studied to the end. Therefore, the aim of the study was to determine the main groups of abnormal seeds, the factors affecting their formation and the characteristics of the germination of such seeds, compared to healthy ones.

MATERIAL AND METHODS

The research was conducted in the conditions of the North-East of Ukraine. The climate of the region is moderately continental. Winter wheat (Bohdana variety) has been growing for five years on experimental fields in the conditions of the Research and Production Complex of the Sumy National Agrarian University. It is located in the Middle Russian forest-steppe region, the Sumy Slope-Upland Region, the ninth physiographic district. The amount of precipitation is moderate (593 mm per year). The average daily annual air temperature is 7.4 °C. The topography of the experimental field is flat. The soil is a typical deep, low-humus, medium-loam chernozem (black soil) on the loess (Neshataev, 2005). In 2017 and 2020–2021, wheat crops were harvested in the city of Shostka (homestead). According to the physical and geographical zoning, this territory is part of the mixed-forest coniferous-broad-leaved moist, moderately warm zone of the Polish province of Novgorod-Siverskyi Polissia. Turf-medium and strongly podzolic sandy soils on alluvioglacial sands and sandy loams prevail here. The average annual air temperature over the last decade has increased to 7.6±0.7 °C, and the annual precipitation has decreased to 629±93 mm (Neshataev, 2005).

The analysis of seeds was done according to macrocharacteristics, as they are often used

in the current standards of Ukraine to determine the quality of seed and grain. The degree of blackening of the germ was determined according to the modified Tropova scale (1955), which is used in Ukraine according to the new DSTU 3768:2019 “Wheat specifications”. Four samples of 100 seeds were taken. Features of germination of different groups of seeds were carried out in a closed room. Five seeds were sown in one cup with soil. 50 pieces of each group of seeds were analyzed in triplicate. Spraying of plants was carried out in the flowering phase of winter wheat with a manual sprayer. A small-scale experiment was established in triplicate (Tribel *et al.*, 2001). The following preparations were involved in the study: Falcon 460 EC, c.e. (emulsifiable concentrate) (tebuconazole 167 g·l⁻¹ + Triadimenol 43 g·l⁻¹ + spiroxamine 250 g·l⁻¹) with a rate of consumption of 2 ml·l⁻¹; Immunocytofit, tb (tablet) (arachidonic acid ethyl ester) – 2 tab·l⁻¹; Trykhotfit, s. (aqueous suspension) (spores of *Trichoderma lignorum*, titer –2.0 bln. cells·cm⁻³) – 25 ml·l⁻¹; Haupsin, s. (aqueous suspension) (bacteria *Pseudomonas aureofaciens* Kluver, strains B-306 and B-111, titer – not less than 10 bln. cells·cm⁻³) –20 ml·l⁻¹; Chitosan, tb. (tablet) (Elite-Pharm LLC, Ukraine) – 3 tab·l⁻¹; essential oils of cinnamon (1.5 ml·l⁻¹) and fennel (2 ml·l⁻¹) (Aromatika LLC, Ukraine). In the control, the plants were treated with water. After harvesting, a macroanalysis of the grain was carried out. Statistical analysis of the results was performed by the method of one-way Anova in Statistica 10.

RESULTS AND DISCUSSION

Factors influencing seed appearance

An external examination of the seeds over several years of research made it possible to distinguish four main groups of winter wheat seeds: healthy, with a black germ, wrinkled to varying degrees, and small. Sometimes isolated cases of seeds with a pink tint were noted. In a previous study, it was established that the presence of these groups is influenced by the genotype of the culture. In 2017, the range of black point manifestation was noted from 0.3 to 19%, the number of wrinkled seeds was in the range of 1.3–21%, small – 0.3–8% in 33 samples of winter wheat from different countries (Rozhkova *et al.*, 2018). We decided to identify other factors that determine the presence of certain groups of seeds in wheat samples. The growing conditions of winter wheat had a significant effect on the manifestation of blackening of the embryo, the number of incomplete and wrinkled wheat seeds (Table 1)

Among seeds of the Bohdana variety, over the past five years in the conditions of the forest-steppe, the largest number of substandard seeds was noted in 2022. Incomplete seeds prevailed among them, although the highest rate – 9.3% was noted among wrinkled seeds. The weight of 1000 seeds was correlated with the percentage of low-quality seeds only in the last years in the Forest- steppe, when the dominance of immature and wrinkled seeds was observed (first three years $r = 0.1$; last three years $r = -0.7$). In 2020, the

Table 1. Results of macroanalysis of seeds (Bohdana variety, Northeastern Ukraine)

Years	Low-quality wheat seeds, %				Weight of 1000 seeds, g
	Black point	Small	Wrinkled	In total	
Forest-steppe					
2017	5.3	8.8	4.0	16.0	49.8
2018	2.0	5.8	1.3	9.1	52.9
2019	1.3	2.0	1.0	4.3	48.9
2020	0.5	6.0	9.3	15.8	39.0
2022	3.5	7.5	7.3	18.3	41.9
Average	2.5	6.0	4.6	12.7	46.5
LSD ₀₅	1.3	2.1	1.8	3.1	6.0
Polissia					
2017	5.3	3.3	4.3	12.9	44.2
2020	17.5	3.0	4.8	25.3	49.4
Average	11.4	3.2	4.6	19.0	46.8
LSD ₀₅	4.1	-	-	7.3	4.2

maximum number of abnormal seeds was noted in Polissia – 25%. The highest rate of black point was noted in this zone for a high weight of 1000 seeds, which indicates the fact of the formation of filled seeds with darkening in the conditions of the North-East of Ukraine. That is, even indirectly, it is possible to assert the fact of a significant presence of *Alternaria* sp. in seeds with a black point. When plants are infected with *Bipolaris sorokiniana* (Sacc.) Shoemaker, weak seeds develop according to Peresyphkin (2000). In addition, the place of cultivation had its influence on the selection of these groups of seeds during macroanalysis, with the exception of the appearance of a black point and the number of wrinkled seeds in 2017.

The manifestation of the black germ in the conditions of the Northeast had its own characteristics. We analyzed this feature on a three-point scale and determined that its significant presence was described in the majority by a score of 1 (Table 2). Moreover, the grain with a colored germ is classified only for the combination of features 1 with 2 or 3. About 60% of the grain, which has a blackened germ only on top, does not belong to this type. That is, in the conditions of the North-East of Ukraine, only 40% of the blackening of the embryo can be attributed to seeds with a black point. Spraying winter wheat during the flowering period has an effect on macrocharacteristics, in addition to the variety, vegetation conditions, and the place of cultivation. This fact was established

by studying the effect of treatments on the formation of seed mycobiota. Wheat spraying reduced the total percentage of abnormal wheat seeds (Table 3). The most effective spraying reduced the number of wrinkled seeds. Trykhofit and Chitosan best reduced the number of low-quality seeds. In the variants with Haupsyn and Immunocytofit completely wrinkled seeds were also not noted. Opposite results were obtained regarding the manifestation of the black point. Only Chitosan, Trykhofit, and Haupsin reduced the number of seeds with this feature. All other substances increased the number of colored seeds, especially essential oils. That is, we have for the first time recorded the fact of the increase of black point after spraying plants with essential oils of fennel and cinnamon.

Germination of healthy and abnormal seeds

In 2018, the seeds were germinated in April with an average air temperature of 12.2 °C. In 2019, the experiment was started in September, when the temperature was 9.7 °C. Since the temperature was low, abundant watering ensured the soil was over moistened. Features of the germination of various seeds are given in Table 4.

The identified group of outwardly healthy seeds germinated better than the others. Moreover, seed germination was slightly better in 2018, and seedling development in 2019. Incomplete seeds

Table 2. Manifestation of the black point seeds (Bohdana)

Place of cultivation	Harvest year	Percentage of seeds with the corresponding score, %		
		1	2	3
Polissia	2020	57	30	13
Forest-steppe	2022	63	35	2

Table 3. The effect of spraying on the amount of substandard seeds (Bohdana, SNAU, 2020)

Variant	Non-standard wheat seeds, %			
	Black point	Small	Wrinkled	In total
Control	0.5	7.5	10.3	18.3
Falcon 460 EC, c.e.	1.3	5.8	1.3	8.4
Haupsin, s. (2 sprays)	0.3	3.5	0	3.8
Immunocytofit, tb. (2 sprays)	3.3	4.8	0	8.1
Trykhofit, s. (2 sprays)	0.3	1.5	0	1.8
Chitosan, tb (3 sprays)	0	2.3	0.5	2.8
Cinnamon (EO)	6.0	1.8	1.0	8.8
Fennel (EO)	9.3	2.3	2.0	13.6
$P_{0.05}$	<0.05	<0.05	-	<0.05

Table 4. Features of germination of externally healthy and abnormal seeds (Bohdana of the 2018 and 2019 harvests)

Indicators	Healthy seeds	Black point	Small seeds	Wrinkled seeds	LSD ₀₅
Seed germination on the 7th day, %	86	58	38	36	4.9
	86	46	64	48	3.9
Seed germination on the 14th day, %	98	76	66	56	4.5
	92	54	72	60	3.7
The length of seedlings on the 7th day, cm	7.0	4.3	5.8	5.7	-
	9.4	9.9	8.3	8.2	-
The length of seedlings on the 14th day, cm	18.9	14.9	9.2	10.5	4.0
	22.5	18.3	14.2	17.5	1.6
Mass of seedlings, g	6.70	4.18	2.58	2.87	0.5
	7.64	5.13	7.61	7.42	0.9

had higher indicators of germination and seedling length in the first seven days of development, compared to wrinkled seeds. But on the 14th day, the seedlings from the latter developed better. As for the germination of black point seeds, contradictory results were obtained. In 2018 their germination prevailed, then in 2019 they germinated the worst from substandard seeds. Seedling length was the lowest in 2018 and exceeded the control in 2019. Only seedling length on the 14th day was the best in both years compared to shrivelled and small seeds. Therefore, we decided to focus further on the determination of the effect of the black point on seed germination in more detail. In 2020–2021, the influence of black germ on the

germination characteristics of winter wheat under conditions of optimal moisture was studied. The experiments included seeds after the storage period, from different places of cultivation and another variety (Table 5).

In 2020, the seeds were germinated at high air temperature in September (the average temperature during the study period was 18.9 °C). The seed germination turned out to be greater during the storage period of one year than during two. In addition, this indicator was higher in seeds with a black point. Better plant development was noted from seeds with a black germ than from healthy seeds in two samples of the studied seeds. In 2021, they investigated the characteristics of seed germination on two different

Table 5. Seedling length and germination of wheat seeds (September 2020, 2021)

Year of harvest/year of analysis T, °C	Indicators	Healthy seeds	Black point	LSD ₀₅
Bohdana (Forest-steppe)				
2018/2020 18.9	Seed germination on the 7th day, %	70	80	7.9
	Seed germination on the 14th day, %	74	82	6.3
	The length of seedlings on the 7th day, mm	78.5	98.9	3.3
	The length of seedlings on the 14th day, mm	255.9	263.7	2.6
2019/2020 18.9	Seed germination on the 7th day, %	88	96.7	4.6
	Seed germination on the 14th day, %	90	96.7	4.6
	The length of seedlings on the 7th day, mm	112.6	121.6	5.4
	The length of seedlings on the 14th day, mm	273.8	284.4	4.9
Bohdana (Polissia)				
2020/2021 11.9	Seed germination on the 7th day, %	90	100	5.2
	Seed germination on the 14th day, %	100	100	-
	The length of seedlings on the 7th day, mm	93.3	100.6	2.9
	The length of seedlings on the 14th day, mm	167.6	171.8	3.4
Lira Odeska (Polissia)				
2021/2021 11.9	Seed germination on the 7th day, %	96	100	3.2
	Seed germination on the 14th day, %	98	100	1.1
	The length of seedlings on the 7th day, mm	95.7	98.9	3.0
	The length of seedlings on the 14th day, mm	191.5	188.3	-

varieties that had a high percentage of black point. The average temperature in September during the experiment was 11.9 °C. Seed germination and length of seedlings turned out to be better in the variant with a blackening, except for the indicator of seedling development on the 14th day on Lira Odeska. Therefore, under favorable germination conditions, seeds with a black point germinated better than healthy ones. Perhaps this fact is explained by the better fullness of the first seed group.

Seed sample of the 2020 harvest with a large amount of black point, grown in the conditions of Polissia, was decided to be analyzed for germination during November–December in an unheated room with an average air temperature during the study period of -0.7 °C. In recent years, late sowing of winter wheat is practiced in connection with climate change in Ukraine, so it was appropriate to conduct such an experiment. At this temperature, the seeds germinated on the 13th day after sowing (Table 6). Seed germination on the 21st day after sowing was 100% for healthy seeds and 96% with a black point, but on the 28th day, their 100% germination was noted. Seedlings first developed faster from healthy seeds, but on the last two measurement dates, they began to grow faster from seeds with a black point.

Grain macroanalysis is a common practice, but its results are subjective. Small, shriveled and black seeded seeds were isolated in this study. The analysis of seeds, which was carried out earlier (2011–2013) in Zhytomyr Polissia, made it possible to distinguish the following groups of diseased grain: smut, grain with signs of *Fusarium* infection and black point (Timoshchuk, 2014). But the range of variation of these traits turned out to be greater by year than by culture genotype. The appearance of the wheat grain is determined by the climatic conditions of the growing region. Thus, in India, wrinkled, black-seeded, and discolored (pink, dark, and white-striped) seeds were identified among abnormal seeds (Pathak and Zaidi, 2013).

Studies on the effect of seed size on future yield have varied results, but recent studies in

Ukraine have shown a positive correlation between size, energy, germination and 1000 seed weight (Skrypnyk et al., 2021). The correlation coefficient between the proportion of abnormal seeds and the mass of 1000 seeds was -0.6, which indicates an inverse relationship between these indicators. Seeds with a black point were in most cases full, which confirmed the fact of its significant presence in samples with a high weight index of 1000 seeds.

The conditions of the growing year determined the presence of certain seed fractions based on its visual analysis. The study by Q.Y. Li *et al.*, (2019) made it possible to identify a number of meteorological conditions of different years for the manifestation of embryo blackening. In Northern India, a study of the development of the black point on different ten varieties during 2016–2017 showed fluctuations in its percentage within the range of 4.2–13.3% (Sharma *et al.*, 2021). L.M. Golosna (2021) showed that in Central Ukraine in 2018, on 27 genotypes, the black point ranged from 0–10.7%, in 2019 its percentage was higher – 0–19.8%. The variety had a greater influence on the manifestation of this trait than the conditions of the year of cultivation. According to the results of different scientists, the effect of black point on seed germination is ambiguous. Fungi, which are isolated from seeds with blackened embryos, have a negative effect on seed germination and the development of wheat seedlings. In the conditions of the North-East of Ukraine, various fungi have been isolated from seeds with a black point, but most of them are *Alternaria* sp. (Rozhkova, 2019). Therefore, under optimal germination conditions, we obtained high rates of seed germination and seedling length, which exceeded healthy seeds. In Central Ukraine, 13 species from nine genera were isolated, among which *Alternaria* sp. also dominated (Holosna, 2021). Energy and germination were high and ranged from 90–100 and 95–100%, respectively. In Northern India, the following fungi were mostly associated with black point: *Alternaria triticina*, *B. sorokiniana*, and *F.*

Table 6. Length of wheat seedlings at low temperature of seed germination (Bohdana, November–December 2020)

Parameter	The length of plants for a certain day, mm				
	21st day	28th day	37th day	46th day	54th day
Healthy seed	19	50.9	86.5	97.7	113.2
Black point	18	50.1	85.2	98.3	115
LSD ₀₅	0.9	-	-	-	1.9

graminearum (Sharma *et al.*, 2021). Therefore, the negative influence of the darkening of the embryo on the germination of seeds and the development of wheat seedlings was noted. If outwardly healthy seeds had 0–12% ungerminated seeds, then with strong darkening – 20–75% (Rani and Sharma, 2018). The similarity of seeds with a black point on filter paper on average for different varieties of durum wheat turned out to be low at about 44%, which was mostly explained by the significant presence of *Fusarium* sp. (Khlebova *et al.*, 2019). A decrease in germination and seedling development from seeds with blackened embryos was noted in China (Li *et al.*, 2014). The main fungi isolated from these seeds were *B. sorokiniana*, *Alternaria alternata* (Fr.) Keissl., and *Fusarium equiseti* (Corda) Sacc. (Xu *et al.*, 2018).

In the study, spraying the plants led to an improvement in the appearance of the seeds: the number of abnormal seeds was reduced. Treatment of seeds and spraying of plants reduces the number of grain with blackened embryos in field experiments (Wang *et al.*, 2002; Paradeshi *et al.*, 2008; Malaker and Mian, 2009; Iqbal *et al.*, 2014). We have shown for the first time that essential oils are able to increase the amount of grain with a black point. This fact once again confirms that fungi are not the cause of blackening.

CONCLUSIONS

In the conditions of the North-East of Ukraine, three groups of abnormal winter wheat seeds were identified by means of macroanalysis: wrinkled, incomplete and with a black point.

The presence of each group is determined by the genotype of the crop (shown by a previous study), the place of cultivation, the growing conditions of the crop and the spraying of plants during flowering. Among the seeds of the Bohdana variety during the last five years in the conditions of the Forest-steppe, the largest number of substandard seeds was incomplete, although the highest rate – 9.3% was noted among wrinkled seeds. The largest number of black point was noted in Polissia (17.5%). Most of the blackened seeds were filled. The analysis of the degree of coloring proved that 60% of the seeds have blackening in the area of the embryo only on top. Only about 40% of the grain can be classified as with a black point. Spraying wheat reduced the total percentage of abnormal wheat seeds, especially

wrinkled wheat. Trykhotit and Chitosan had the highest efficiency. For the first time, an increase in black point was recorded after spraying plants with essential oils of fennel and cinnamon. By comparing the germination of non-condensed and healthy seeds, better indicators were found for the latter, with the exception of the black point, which determined the further direction of research. Comparison of seed germination and development of seedlings from healthy and blackened seeds showed better results of the second group at high temperature and optimal humidity in September. An experiment at a low temperature of late sowing showed faster germination and better development of seedlings from healthy seeds. Therefore, the similarity of different groups of seeds and the development of seedlings is determined by the growing conditions (air temperature, soil moisture, and photoperiod).

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