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The influence of MGA and NS media over the isolation of *Fusarium* colonies from infected wheat seeds

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Abstract Several species of the genus *Fusarium* are involved in Fusarium Head Blight (FHB), also known as "scab," a widespread disease which can reduce the crop yield up to 80% of production. Therefore, permanent monitoring required rapid isolation of infectious species using an appropriate medium. In our research we use two media for isolation of *Fusarium* species that infects wheat crops in the Timiş County, MGA and NS, establishing by analysis of variance as the best medium to achieve significant results for the isolation of the different species of *Fusarium* is MGA.

Key words

Fusarium, wheat, seeds, colonies

Whereas species of the genus *Fusarium*, except *F.culmorum* are weak competitive compared to other types of fungi, such as *Penicillium* and *Aspergilus,* it is important to use a selective medium that allows the development of *Fusarium* species, but which exerts an inhibitory effect on others (Windels, 2000). The most commonly used selective media for *Fusarium sp*, are PPA (PCNB Peptones Agar), Komada (Komada, 1975) and NS (Nash and Snyder). These three media contain pentachloronitrobenzene (PNCB), substance with strong inhibitory action on most fungi, except *Fusarium sp*. Since it was discovered carcinogenic effect of PNCB (IARC 1987a, 1987b), these media are less used, being replaced by MGA (Leslie and Summerell, 2006). Develeped in 1997 by Castella et al., MGA (Malachite Green Agar) medium contains malachite green as inhibitory substance, being more inhibitory of *Aspergilus* and *Penicillium*, without reducing the number of colonies of *Fusarium sp.* (Castela et al., 1997; Alborch, 2010).

Material and Methods

Biological material used in our research is Alex-variety of wheat grown in different areas of Timiş County. It is a variety with an average resistance to *Fusarium* and received proper cultivation technology. Wheat samples were named after the localities where they were collected from. In this way were collected 30 samples of infected seeds, each sample containing about 10-14 seeds of wheat with obvious symptoms of infection. Following recommendations from the literature (Castela et al., 1997) were used MGA and NS media to isolate strains of *Fusarium* from infected seeds.

Data obtained from different analyzes were processed statistically, determining the average, standard deviation and coefficient of variance.

- average: $\bar{x} = \frac{\sum x}{n}$; - average error $s_{\bar{x}} = \frac{1}{n}\sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$; - coefficient of variation: $s_{\%} = \frac{s \times 100}{\bar{x}}$

To determine the significance of differences between studied genotypes, processing of experimental data was realised by analysis of variance and t test for mono-factorial experience, two-factorial of type 2 x 16 (localities x media) three-factorial 2 x x16 (Species x Media x localities) after Ciulca S. (2006).

The significances of differences were represented both on the basis of symbols (*, *, * I *; ^{0}; ^{00}; ^{000}) and based on the letters, being considered significant differences between variants denoted by different letters.

Results and Discussions

The results presented in table 1 indicates that both the culture medium used and the environmental conditions in the various localities, have a real influence on the number of distinct colonies of *Fusarium* isolated from infected seed. The composition of the culture medium has the most significant influence (90,81) on the development of the colonies, while the origin of the seeds, and growing conditions in which they were obtained showed a statistically assured contribution of 9,19% of the infestation with *Fusarium*. It was also observed that the combined

effects of those two factors have had significant influence (as 5.20 percent), distinctly significant degree of contamination of the seed.

Table 1

Variance analysis of the effect of the culture and ecological conditions on the number of colonies of *Fusarium* isolated from seeds

Source of variation	SP	GL	S^2	Test F
Total	942,44	299		
Culture medium	105,76	1	105,76	F =72,44**
Localities	310,54	29	10,71	F =7,33**
Media x Localities	175,74	29	6,06	F =4,15**
Erorr	350,40	240	1,46	

The results presented in Table 2 show that the number of colonies isolated from seeds showed a very high variability of values, ranging between 2.68 for NS and 4.17 for MGA. Amid this variability it can be affirmed that MGA medium has allowed a very notable increase in the number of colonies *Fusarium*, with approximately 55%. So MGA medium can be used with high degree of efficiency to select *Fusarium* colonies.

Table 2

The number of *Fusarium* colonies isolated from seed in different culture media

Culture medium	Number of colonies		Values relatives(%)	Diference/ Semnification
NS - MGA	2,68	4,17	64,27	$-1,49^{000}$

$DL_{5\%}$=0,28 $DL_{1\%}$=0,36 $DL_{0,1\%}$=0,47

Table 3

The number of *Fusarium* colonies isolated from seeds originating from different localities of Timis County

Nr. crt.	Locality	Number of colonies $\bar{x} \pm s_{\bar{x}}$	Number of colonies $s_{\%}$	Value relative (%)	Semnification of diferention
0	1	2	3	4	5
Media experienţei		3,42±0,10	51,16	100	Martor
1	Birda	3,50±0,62efghij	55,94	102,34	0,08
2	Gataia	2,10±0,62mn	93,77	61,40	$-1,32^{0}$
3	Pădureni	3,90±0,35defgh	28,22	114,04	0,48
4	Obad	4,20±0,63cdef	47,35	122,81	0,78
5	Parţa	4,30±0,30bcde	22,06	125,73	0,88
6	Carani	3,10±0,38ghijklm	38,62	90,64	-0,32
7	Sînnicolau Mare	2,90±0,38hijklmn	41,28	84,80	-0,52
8	Coştei	2,20±0,20lmn	28,75	64,33	$-1,22^{0}$
9	Giroc	3,40±0,34efghij	31,62	99,42	-0,02
10	Pietroasa	5,30±0,21ab	12,73	154,97	1,88***
11	Criciova	4,90±0,60abcd	39,02	143,27	1,48**
12	Ghilad	2,30±0,63klmn	87,08	67,25	$-1,12^{0}$
13	Balinţ	5,70±0,40a	21,96	166,67	2,28***
14	Boldur	3,60±0,40defghij	35,14	105,26	0,18
15	Soca	4,00±0,30defg	23,57	116,96	0,58
16	Sînmihaiu Român	3,40±0,72efghij	66,78	99,42	-0,02
17	Lugoj	2,90±0,23hijklmn	25,44	84,80	-0,52
18	Macedonia	5,10±0,38abc	23,47	149,12	1,68**
19	Gavojdia	0,40±0,16o	92,10	11,70	$-3,02^{000}$
20	Ionel	2,10±0,31mn	47,35	61,40	$-1,32^{0}$
21	Ciacova	4,60±0,27bcd	18,33	134,50	1,18*
22	Bethausen	2,80±0,47ijklmn	52,70	81,87	-0,62
23	Orţisoara	3,40±1,02efghij	95,25	99,42	-0,02

0	1	2	3	4	5
24	Uivar	4,30±0,45bcde	32,98	125,73	0,88
25	Timişoara	2,60±0,31jklmn	37,16	76,02	-0,82
26	Opătiţa	3,70±0,26defghi	22,25	108,19	0,28
27	Găvojdia	3,20±0,25fghijkl	24,65	93,57	-0,22
28	Biled	3,30±0,50efghijk	47,49	96,49	-0,12
29	Jimbolia	3,60±0,31defghij	26,84	105,26	0,18
30	Cenei	1,90±0,23n	38,83	55,56	$-1,52^{00}$

$DL_{5\%}$=1,07 $DL_{1\%}$=1,41 $DL_{0,1\%}$=1,80

Regarding the origin of analyzed seeds, their infestation showed a 3 magnitude, due to a variability of 38.83% between different localities, with values between 0.4 colonies for seeds collected from Gavojdia and 5.70 for those collected from Balint. Ecological conditions in the studied period generated a high variability in the level of infestation of seeds for each analyzed locality. *Fusarium* contamination of seeds throughout the experiments showed a distribution with a high degree of symmetry. Thus, the largest share amount of 40% was find in localities where infestation degree was 3-4 colonies, while 26% of villages have been identified 2-3 colonies, respectively 4-5 colonies in the case of the 20% of locations.

Table 4

The effect of culture medium and environmental conditions from different localities on the number of *Fusarium* colonies isolated from seeds

Nr. crt.	Locality	Culture medium	
		MGA	**NS**
1	Birda	x5,20abcde	y1,80ghi
2	Gătaia	x3,80efgh	y0,40i
3	Pădureni	x4,20cdefgh	x3,60cde
4	Obad	x6,00ab	y2,40defg
5	Parţa	x4,80bcdef	x3,80bcd
6	Carani	x4,00defgh	x2,20efg
7	Sînnicolau Mare	x3,80efgh	x2,00fgh
8	Coştei	x2,20ij	x2,20efg
9	Giroc	x4,20cdefgh	x2,60defg
10	Pietroasa	x5,40abcd	x5,20ab
11	Criciova	x6,40a	y3,40cdef
12	Ghilad	x4,00defgh	y0,60hi
13	Balinţ	x5,00abcdef	x6,40a
14	Boldur	x4,60bcdefg	y2,60defg
15	Soca	x3,60fghi	x4,40bc
16	Sînmihaiu Român	x5,40abcd	y1,40ghi
17	Lugoj	x3,20ghij	x2,60defg
18	Macedonia	x5,60abc	x4,60bc
19	Găvojdia	x0,40k	x0,40i
20	Ionel	x1,80jk	x2,40defg
21	Ciacova	x4,80bcdef	x4,40bc
22	Bethausen	x4,00defgh	y1,60ghi
23	Orţisoara	x6,40a	y0,40i
24	Uivar	x5,00acdef	x3,60cde
25	Timişoara	x3,20ghij	x2,00fgh
26	Opătiţa	x4,00defgh	x3,40cdef
27	Gavojdia	x2,80hij	x3,60cde
28	Biled	x4,60bcdefg	y2,00 fgh
29	Jimbolia	x4,40cdefg	x2,80 defg
30	Cenei	x2,20ij	x1,60 ghi
	$\bar{x} \pm s_{\bar{x}}$	4,17±0,13	2,68±0,13
	$S_{\%}$	37,66	59,95

-Averages - $DL_{5\%}$=1,51 $DL_{1\%}$=199 $DL_{0,1\%}$=2,55 (x,y)
-Localities- $DL_{5\%}$=1,51 $DL_{1\%}$=199 $DL_{0,1\%}$=2,55 (a,b,c)

Compared to the average, approximately 46% of the localities have registered a higher level of infestation with *Fusarium*, but in the case of the samples collected from Balinţ (2,28 * * *), Pietroasa (1.88 * * *) and Macedonia (1.68 **), there was a significant increase in the number of colonies isolated from seeds, with 49-66%. Amid the environmental conditions in the localities: Găvojdia (-3,02^{000}), Cenei (-1.52^{00}), Gătaia (-1,32^{0}), Ionel (-1,32^{0}), Coştei (-1.22^{0}) and Ghilad (-1,12^{0}), *Fusarium* attack was manifested at a considerably reduced level 33-88% compared to overall average experience.

Based on multiple comparisons it was noted that samples collected from Balinţ showed a degree of contamination significantly higher than the 93% of the analysed samples. Also, the environmental conditions of Pietrosa and Macedonia have favored a significant increase of *Fusarium* attack compared to 73% of the locations included in this study. In ecological conditions of Găvojdia, it was found an infestation level significantly below the results collected from the rest of the localities included in this study.

Regarding the interaction between origin and seed culture medium (Table 4; Figure 1) our study revealed that the highest differentiation between varieties manifested NS environment, while under the effect of culture conditions on MGA, seed samples recorded lower amplitude of colonies number. It also noted that the MGA samples from Orţişoara and Criciova showed a significantly higher number of colonies compared with 75% of the remaining studied samples, while on the medium NS highest values of the number of colonies were isolated from samples from Balint and Pietroasa. Given the low values of seed contamination in samples collected from Gavojdia it was observed that the number of identified colonies on the two culture media was constant.

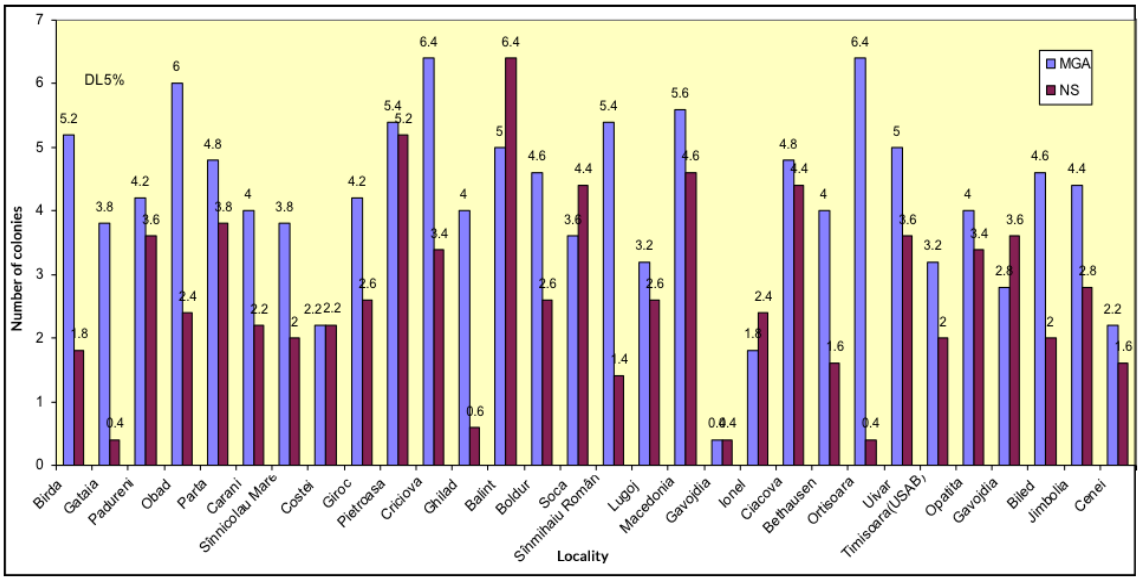

Fig. 1 Number of Fusarium colonies isolated on different culture media from infected seeds collected in Timiş County

In the case of 66% of the analyzed samples, the composition of the culture medium had a little and insignificant influence over the isolation of *Fusarium* colonies. The culture medium had the highest influence on the development of *Fusarium* colonies in samples from localities: Orţişoara, Gătaia and Ghilad. In the case of samples from Gavojdia, Ionel and Soca, on NS were isolated a greater number of colonies, but this difference is not statistically assured.

Based on these results we can say that MGA medium allows the development of fungi of the genus *Fusarium* in a higher percentage than the NS medium. These results are similar to those published in the literature, emphasizing the high selection potential of MGA medium. Thus, studies of Alborch et al. (2010) on a sample of 10,800 cereal grains pointed out the upper selection potential of MGA (72.1%) compared with NS (65.5%) for the development of *Fusarium* strains.

Conclusion

We conclude that the results obtained in our research, harmonize with those from the literature, the higher selective potential of MGA medium being obvious.

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