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Accessing the relevance of tests for estimating the physiological quality of wheat grains

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Abstract: The objective of this work was to investigate, before and after chemical treatment, the interrelationships of the physiological quality tests in wheat grains. Following the chemical treatments with (*i*) carboxin thiram; (*ii*) carboxin thiram + thiamethoxam; (*iii*) carboxin thiram + thiamethoxam + bio-stimulant; (*iv*) pyraclostrobin + methyl thiophanate + fipronil, and (*v*) pyraclostrobin + methyl thiophanate + fipronil + bio-stimulant, subsequently the grains were assessed for their physiological potential. The physiological potential of the grains could be differentiated based on the electrical conductivity, seedling growth, and emergence in sand tests. In conclusion, the use of the active ingredients changed the correlation between laboratory tests with crop emergence in the sand as well as altered the relevance of the tests for sorting the treated grains into different physiological potential. But, the seedling growth tests and the electrical conductivity were, together, the parameters most relevant for explaining the data variability.

Keywords: agrochemicals; Pearson; active-principle; *Triticum aestivum* L.

The evaluation of the physiological potential of crop seeds should allow a reasonable forecast of the performance of one lot in the field (Marcos-Filho 2015). For this, in addition to the germination test, which evaluates the maximum potential of seed to generate normal seedlings, the use of vigor tests is considered essential, as they evaluate different attributes of the seeds that interfere in the probability of one lot to provide the target plant stand (Marcos-Filho 2015). For the International Seed Testing Association, vigor is the "total sum of the properties of seeds that determine the activity and performance of seed lots with acceptable germination in a wide range of environments" (ISTA 1995).

For wheat, Abati et al. (2018) reported that high vigor grains favor the initial stand, growth, and development of the plants in the initial phenological stages, as well as grain yield. In crops such as maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merrill.), the radicle emission test for the first species and the electrical conductivity, tetrazolium, and the accelerated aging tests for the second one has validated vigor assessments requested for seeds moving in international trade (ISTA 2017). However,

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the wheat (*Triticum aestivum* L.), one of the main cereals grown worldwide, still lacks vigor tests internationally accepted as capable of providing reliable and reproducible results (ISTA 2017).

This is the reason why in commercial production of wheat grain, the quality control system often subjects the lots to numerous tests in order to obtain a reliable status of their physiological potential. In these conditions, if the tests are used to evaluate the same vigor attribute (Lorentz and Nunes 2013), they may provide redundant results and, thus, too little contribute to sort the lots into different physiological potential. Furthermore, sowing rarely occurs in areas free from pests or phytopathogens, requiring that the seeds be treated with the pesticides (Suzukawa et al. 2019). These products have already reported in the literature as harmful to the grain vigor of wheat (Abati et al. 2014, Baldini et al. 2018), and, in the case of maize, were already pointed out capable of interfering with the accuracy of physiological tests to estimate the crop emergence in the field (Pereira et al. 2019).

In this scenario, the hypothesis established in this work is that, by affecting the physiological potential of wheat grains, chemical treatment alters the precision and relevance of physiological quality tests to predict the emergence of wheat grain. Thus, the objective of this work was to investigate, before and after a chemical treatment, the interrelationships of the physiological quality tests of wheat grains commonly employed in the production of this cereal.

MATERIAL AND METHODS

Two wheat cultivars were used in the study: Cv. TBIO Toruk, of industrial suitability and germination of 93%, and cv. Energia, used for the production of silage and germination of 87%. The grains were manually treated with the pesticides described in Table 1. As a control, grains without any treatment were used.

The evaluation of the physiological potential of the grains was carried out by means of the tests of germination (MAPA 2009), first germination count (MAPA 2009), accelerated aging (ISTA 2017), electrical conductivity (AOSA 2002), modified cold test (Pereira et al. 2019), emergence in the sand test (Pereira et al. 2019), dry seedling mass (Nakagawa 1999), as well as seedling growth tests (shoot length, root length, and total seedling length), according to the method described by Abati et al. (2014).

The experiment was conducted by adopting a completely randomised design with treatments arranged in a 6 × 2 factorial scheme: six treatments (without and with pesticides) and two commercial wheat cultivars (TBIO Toruk and Energia), with four replicates each. The data obtained were initially tested for normality (Shapiro-Wilk) and homogeneity of residual variances (Levene). Then, the parameters of physiological potential were subjected to the two-way ANOVA ($P \leq 0.05$) variance analysis and, when significant, the means were compared to the control by means of the Dunnett's test ($P \leq 0.05$) using the SISVAR system for statistical analysis (Ferreira 2014). Then, in order to find out how the chemical treatment interfered in the physiological potential of wheat grains, the data of all tests were subjected to Pearson's correlation with the results of seedling emergence in the sand substrate, which among the tests carried out, it is the closest one to mimic the environmental conditions that the crop may find in the field. Finally, in order to elucidate which of the tests employed are more capable of explaining the variability of the results, the main component analysis was adopted according to Johnson and Wichern (2005).

Table 1. Summary scheme of the treatments applied to the wheat grain

Treatment	Products used in the treatments	Syrup volume (mL/100 kg)
Control	untreated grains	–
F	fungicide (250 mL)	250
FI	fungicide (250 mL) + insecticide (250 mL)	500
FIB	fungicide (250 mL) + insecticide (250 mL) + bio-stimulant (600 mL)	1 100
F/I	fungicide/insecticide (200 mL)	200
F/I + B	fungicide/insecticide (200 mL) + bio-stimulant (600 mL)	800

F – fungicide: [carboxin (200 g/L) + thiram (200 g/L)]; I – insecticide: [thiametoxam (350 g/L)]; F/I – fungicide/insecticide: [pyraclostrobin (25 g/L) + methyl thiophanate (225 g/L) + fipronil (250 g/L)]; B – bio-stimulant: [kinetin (0.09 g/L) + indolebutyric acid (0.05 g/L) + gibberellic acid (0.05 g/L)]

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Table 2. Mean percentage of the unfoldings of the grain treatment × cultivar first order significant interaction for the following variables: accelerated aging; dry mass, and total seedling length

Grain treatment	Accelerated aging		Dry mass		Total seedling length	
	cv. Toruk	cv. Energy	cv. Toruk	cv. Energy	cv. Toruk	cv. Energy
Control	88.00	71.60	0.133	0.123	24.28	23.62
F	84.60*	65.80*	0.136*	0.115*	24.41*	22.31*
FI	79.50*	57.78*	0.145*	0.109*	25.81*	20.80*
FIB	63.80*	44.40*	0.123*	0.115*	20.62*	21.49*
F/I	85.40*	73.50*	0.139*	0.117*	25.81*	24.51*
F/I + B	65.60*	66.80*	0.131*	0.118*	23.03*	23.03*
CV (%)	20.51		12.30		13.86	

CV – coefficient of variation. *Mean differ from control treatment at 5% of significance level by Dunnett test. F – fungicide; I – insecticide; B – bio-stimulant

RESULTS AND DISCUSSION

In the ANOVA ($P \leq 0.05$), the factors grain treatment and cultivar showed significant interactions for the accelerated aging (AA), dry mass (DM), and total seedling length (TSL) tests, while for the variables germination (G), first germination count (FGC) and shoot length (SL) the pesticides and the cultivars were statistically significant as individual factors. In the modified cold test (MC), seedling length (SES), and electrical conductivity (EC), only the factor cultivar showed significance, whereas the root length had no statistical significance either in the interaction or in the isolated factors.

Regardless of the active ingredient, the chemical treatments were detrimental to the physiological performance of the grains measured by the tests of AA in the cv. Toruk and DM in the cv. Energia (Table 2). However, compared to the control, the F/I treatment provided beneficial effect to the vigor, as observed in the tests of AA (cv. Energia), DM (cv. Toruk) as well as in the TSL for both cultivars. Beneficial effects of the fungicide associated or not with the insecticide (F and FI treatments) on the cv. Toruk grain were observed in the DM and TLS, (Table 2) similar results were reported by Marini et al. (2011) when evaluating the effect of the fungicide [carboxin-thiram] on the physiological quality of CD 111wheat grains.

The percentage of normal seedlings obtained in variable G (Table 3) remained above 80%, the minimum standard indicated for the commercialisation of wheat in Brazil (MAPA 2013). However, differently than the G, a beneficial effect on the physiological po-

Table 3. Mean percentage of the effect of the factors on the germination (G); first germination count (FGC); shoot length (SL); modified cold (MC); seedling emergence in the sand (SES) and electrical conductivity (EC)

		G	FGC	SL	MC	SES	EC
Cultivar	Toruk	92.70[a]	86.60[a]	10.47[a]	91.25[a]	90.38[a]	63.21[a]
	Energia	86.89[b]	80.48[b]	8.66[b]	89.24[b]	85.39[b]	46.45[b]
Grain treatment	control	92.35	89.95	9.67	ns	ns	ns
	F	91.15*	85.45*	9.56*	ns	ns	ns
	FI	89.84*	81.17*	9.52*	ns	ns	ns
	FIB	87.15*	74.00*	8.97*	ns	ns	ns
	F/I	90.65*	86.95*	10.10*	ns	ns	ns
	F/I + B	87.70*	83.75*	9.60*	ns	ns	ns
CV (%)		9.36	12.77	14.33	6.95	7.87	16.59

CV – coefficient of variation. Means followed by the same lowercase letters in the column do not differ from each other by the *F*-test at a 5% probability level. *Means differ from control treatment at 5% of significance level by Dunnett test. ns – no significant effects of chemical treatment; F – fungicide; I – insecticide; B – bio-stimulant

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tential of the grains was observed in the F/I treatment in the SL variable, compared to the control (Table 3). Overall, the greater physiological potential was observed of the grains of the cv. Toruk compared to cv. Energia, as assessed in the G, FGC, SL, MC, and SES tests (Table 3), but contrarily to these tests the first cultivar displayed higher conductivity readings. In the EC test, seeds with damaged or deteriorated membranes, that is, potentially less vigorous, release more exudates to the outside of the cell, which results in higher conductivity values (Marcos-Filho 2015). This rational applies to many agricultural crops; however, wheat grains have been pointed out as an exception since its coating structure (the seed itself as well as the caryopsis).

These results shown in Tables 2 and 3 are consistent with Cunha et al. (2015), who pointed out that the physiological performance of grains varies according to test, the active principles, and the doses of the phytosanitary products used. In this work, to ascertain how the grain treatment interfered in the ability of the physiological assessment for estimating the wheat emergence, the data obtained in each test were subjected to Pearson's correlation test, based on the results of the emergence in the sand (Table 4).

Our data on Pearson correlation corroborate Pereira et al. (2019), who also reported that grain treatment interfered in the relationship between emergence in the sand with the other tests physiological tests in maize. Indeed, based on the correlation values of Table 4, the most suitable test for predicting wheat seedling emergence in the sand varied with chemical treatment.

In Pearson's correlation, the values closer to –1 or 1, presents a higher degree of correlation degree among the variables. Due to the great variability in the level of vigor existing among agricultural species and even among lots of the same crop (Matera et al. 2019), there are no reference values for the interpretation of this coefficient in the area of wheat grain. However, based on the results of other crops such as maize (Pereira et al. 2019), soybean (Matera et al. 2019), and rice (Lorentz and Nunes 2013), in this study, we considered relevant results above 0.599.

In the control treatment, this criterion was found only in the SL variable ($P \leq 0.05$), while in the treatment based on carboxin + thiram (F), significantly relevant values were found in the variables AA and EC. With the addition of thiamethoxam to the fungicide slurry (FI), the variables that displayed coefficient higher than 0.599 were G, CE, DM, RL, and TSL, whereas, in the presence of the biostimulant (FIB) only the G, FGC and CE correlated with SES ($P \leq 0.05$). Regarding the F/I treatment, relevant and significant values were observed only in the variables AA and RL, but when the biostimulant was added (F/I + B), G and FGC established strong relationships with the SES.

To verify the importance of each test in the classification of the vigor, the main component analysis was performed, in which, each physiological test was assessed as to its relevance to explain the variability of the data, that is, as to the proportion of the total variance explained by each of them. For Johnson and Wichern (2005), the components that together explain at least 80% of the accumulated variance should be considered more relevant. In this context, for control, this level was reached by the TSL, EC and AA tests; they appear in the treatment based on the fungicide (F) together with the RL test; for the grains treated with FI, the most relevant tests were EC, FGC and RL (Table 5). With the addition of the bio-stimulant to the slurry (FIB), the profile of main

Table 4. Pearson's estimated linear correlation coefficients between emergence and the answered germination (G); first germination count (FGC); accelerated aging (AA); electrical conductivity (EC); modified cold (MC); dry mass (DM); shoot length (SL); root length (RL), and total seedling length (TSL) variables

	Treatment	G	FGC	AA	EC	MC	DM	SL	RL	TSL
Emergence in sand	control	0.204	0.195	0.566	0.400	–0.309	0.249	0.633*	0.211	0.459
	F	0.616	0.619	0.791*	0.785*	0.472	–0.205	–0.012	–0.628	–0.258
	FI	0.737*	0.615	0.330	0.807*	0.526	0.655*	0.556	0.707*	0.787*
	FIB	0.773*	0.794*	0.121	0.646*	0.305	0.093	–0.135	–0.074	–0.143
	F/I	0.352	0.389	0.788*	0.518	0.007	0.241	0.205	–0.680*	–0.380
	F/I + B	0.730*	0.769*	–0.407	0.567	0.567	0.221	0.353	0.208	0.377

*Statistically significant at 5% of probability; F – fungicide; I – insecticide; B – bio-stimulant

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Table 5. Load accumulated by the parameters germination; first germination count (FGC); accelerated aging (AA); electrical conductivity (EC); modified cold (MC); dry mass (DM); shoot length (SL); root length (RL); total seedling length (TSL), and seedling emergence in sand (SES) variables and their contributions according to the main component analysis

Treatment	Variables	Unit (%)	Total (%)
Control	TSL	39.70	85.20
	EC	30.41	
	AA	15.09	
F	EC	44.20	92.86
	TSL	28.13	
	RL	13.42	
	MC	7.11	
FI	EC	57.64	93.74
	FGC	16.74	
	RL	11.54	
	AA	7.82	
FIB	EC	36.99	93.32
	DM	25.45	
	SL	19.56	
	MC	11.32	
F/I	EC	39.88	92.76
	TSL	31.91	
	DM	12.41	
	MC	8.61	
F/I + B	SES	43.39	86.57
	TSL	26.21	
	DM	16.97	

F – fungicide; I – insecticide; B – bio-stimulant

components altered, with EC, DM, and SL reaching the minimum level of importance (Table 5). For the F/I treatment, however, the main factors were EC, TSL, and DM, while for the F/I + B treatment, they were SES, TSL, and DM.

The results of main components allowed us to state that, although the G or the FGC had a strong correlation with the emergence in the sand in the FI, FIB and F/I + B treatments, these tests were not relevant to explain the physiological potential of the lot (Table 5). Confirming that the potential physiological is a complex of attributes that are interrelated, we observed that, although not effective to estimate the emergence in the sand in the Pearson correlation (Table 4), the TSL test contributed to explain the data variability of the treatments F; F/I and F/I + B (Table 5). Such a same outcome applies to the AA for the FI treatment As well as for the DM test in the FIB treatment (Tables 4 and 5).

Among the tests, the EC was the one to show a strong correlation with the emergence in the sand for the treatments F, FI and FIB (Table 4) and, at the same time, it most contributed to explain the variability of the data on physiological quality (Table 5). But for the treatments F/I and F/I + B, any of the tests that correlated with the emergence (Table 4) was listed in the main component analysis. In conclusion, the use of the active ingredients changed the correlation between laboratory tests with crop emergence in the sand as well as altered the relevance of the tests for sorting the treated grains into different physiological potential. But, the seedling growth tests and the electrical conductivity were, together, the parameters most relevant for explaining the data variability.

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