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RESEARCH ARTICLE

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PHYSIOLOGICAL QUALITY OF MAIZE SEEDS TREATED WITH BIOSTIMULANT, LIQUID FERTILIZER AND *AZOSPIRILLUM BRASILENSE*

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ABSTRACT

This work verified the physiological quality of seeds of two maize cultivars treated in isolation and in association with an inoculant based on *Azospirillum brasilense*, biostimulants and a liquid fertilizer. The experiment was designed in a completely randomized 2x8 factorial scheme, comprising two maize cultivars (P30F53 and P30F53YH) and eight seed treatments (untreated; treated with inoculant; treated with biostimulants; treated with liquid fertilizer; treated with inoculant and biostimulant; treated with inoculant and liquid fertilizer; treated with biostimulant and liquid fertilizer; treated with inoculant, treated with liquid fertilizer and biostimulant). Percentage of germination, emergence in sand, germination speed index, cold test, and dry mass of shoots and root of seedlings were evaluated. The cultivar P30F53YH had seeds with better physiological quality compared with the cultivar P30F53 when treated with biostimulants, liquid fertilizer and *Azospirillum*. The biostimulant, liquid fertilizer and *Azospirilum*. either on their own or in combinations not involving the liquid fertilizer do not impair physiological qualityof maize seeds.

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INTRODUCTION

The use of different products in the treatment of maize seeds, such as fungicides, insecticides, micronutrients, biostimulants, polymers, graphite and more recently biological products through inoculants has become an increasingly common practice (Pedrini *et al*., 2017; Ma, 2019). In this sense, product compatibility assessments, especially involving biological inputs, are essential to identify the effectiveness of products, as possible antagonistic or synergistic effects thatcan occur between formulations (Hungria *et al*., 2010; Hungria *et al*., 2020). Inoculation of microorganisms in seeds, in combination with other synthetic inputs, should ensure the survival of the microorganisms, the efficiency of pesticides and physiological quality ofseeds, allowing the farmersa greater added value to the treated seeds and an increased efficiency in sowing operations. Response to the application of micronutrients, biostimulants and microorganisms inmaize crop is still varied, but the gain in physiological response and productivity that occurs in some cases and, consequently, the relative reduction in production costs have promoted the use of these products in Brazil (Martins *et al*., 2016; Souza *et al*., 2019). Techniques that seek improvements in seed physiological quality are important to boostthe performance potential and stand uniformity in the field (Aragão *et al*., 2003; Itroutwar *et al*., 2020).So, bacteria of the genus *Azospirillum* act primarily in biological nitrogen fixation (Ashraf *et al*., 2011; Zeffa *et al*., 2018), showing the ability to reduce and promote the use of atmospheric nitrogen by plants through biological fixation.On the same hand, *Azospirillumbrasilense*can also stimulate growth and affect plant development by producing phytohormones that promote shoot and root growth, such as indole acetic acid (Moreira *et al*., 2010; Fukami *et al*., 2018) and activities of several enzymes for stimulating germination (Liu *et al*., 2019). Biostimulants, however, are synthetic analogues to plant hormones which act on the processes of degradation of seed reserve substances, cell differentiation, division and elongation, among other physiological processes (Malik *et al*., 2021). Biostimulantsact as activators of cell metabolism, strengthening the immune system, reactivating internal plant processes at different stages of development (Silva *et al*., 2010).

These growth regulators have a close relationship with mineral metabolism in plants, being dependent on the synthesis and availability of certain nutrients (Huang *et al*., 2021) as well as in the synergistic modulation of other processes, such as biological nutrient fixation. The main function of zinc is the activation of enzymes, acting on the synthesis of the amino acid tryptophan, which is the precursor of the biosynthesis of indolacetic acid (IAA), responsible for cell elongation (Ribeiro & Santos, 1996; Prado *et al*., 2007). Also, molybdenum is a determinant factor for biological nitrogen fixation, playing an indispensable role in the assimilation of nitrate absorbed by plants (Favarin *et al*., 2008; Taiz *et al*., 2017). The objective of this work was to evaluate the physiological quality of maize seeds submitted to different treatments involving growth regulators, liquid fertilizer and an inoculant composed of *Azospirillumbrasilense*.

MATERIALS AND METHODS

The experiment was carried out in the seed laboratory at Embrapa Maize and Sorghum, in the municipality of Sete Lagoas, State of Minas Gerais, Brazil. Conventional and transgenic versions of the seeds of two simple hybrids of maize (P30F53) were treated with different products both in combination and in isolation. A completely randomized experiment with 4 replicates was designed, making up a 2 x 8 factorial scheme, comprising two maize genotypes and eight seed treatments. The seed treatments consisting of the isolated application of inoculant (T2), biostimulant (T3); micronutrients (T4); inoculant and biostimulant (T5); inoculant and micronutrients (T6); biostimulant and micronutrients (T7) and the combination of the three products (T8). Additionally, untreated seeds were evaluated as control treatments (T1). The inoculant used, composed of *Azospirillum brasilense* (AzoTotal®) was applied using the dosage of 100 mL of the commercial product $60{,}000^{-1}$ seeds, containing 2×10^{11} UFC l^{-1}. As a biostimulant, the product Stimulate® was used with the dosage of 12.5 mL kg^{-1} of seeds, composed of gibberellic acid 0.05 g L^{-1}, 4-indole-3-butyric acid 0.05 g L^{-1} and kinetin at 0.09 g L^{-1}, with a density of 1.019 g mL^{-1}. Micronutrients were applied to the seeds using a commercial liquid fertilizer (Cellerate®), using 17.5 ml kg^{-1} of seeds of the commercial product. The liquid fertilizer formulation comprises 5% zinc; 10% molybdenum; 11.4% P_2O_5 and 2.3% sulfur with a density of 1.48 g mL^{-1}.

When combined, microorganism inoculation wasthe last treatment, following the application of liquid fertilizer and biostimulant. After manual application and homogenization, seeds were dried in a controlled environment (25ºC) protected from lightuntil sown. Germination test of maize seeds was carried out on sheets of Germitest paper, in rolls moistened with water (Brasil, 2009). The rolls of Germitest paper were kept in germinators at a temperature of 25 °C and the number of normal seedlings was counted at 4 and 7 days, according to the criteria established by RAS (Brasil, 2009). Each experimental plot consisted of 50 seeds, using 4 replicates per treatment. Additionally, the emergence test was carried out on a soil with daily assessments from the start ofemergence, computing the number of emerged seedlings until stabilization. Percentages of normal seedlings at 21 days and the emergence speed index were calculatedaccording to Maguire (1962). In order to determine the effect on seedling vigor under cold conditions, a modified test was carried out on sheets of Germitest paper. Paper rolls were kept in germinators at a temperature of 10 °C for seven days and then at 25 °C for five days, at which point seedlings and non-germinated seeds wereevaluated with results expressed in percentage. Seedling shoot and root dry matter mass were evaluated in the germination test at seven days. Seedlings were manually separated byshoot and root. Seedling parts were arranged in separate paper bags and taken to a forced air circulation oven, regulated at a temperature of 65 °C, until the seedling parts reached constant mass. Dry masses of seedling parts were calculated and expressed in grams per plant. Results were subjected to analysis of variance ($p<0.05$) and Scott-Knott means grouping testwascarried out usingthe statistics software SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

Effect of seed treatments on all evaluated variables was observed, except for the emergence test (ET) and emergence speed index (ESI). Cultivars influenced variables, except for the 2^{nd} germination reading (2^{nd} READ) and shoot dry weight (SDW). Interaction effects were observed for cold test (CT) and root dry weight (RDW) (Table 1). Seeds had a higher germination rate when inputs were used in isolation, showing no difference from control samples. It became evident that the use of liquid fertilizer in association with another product impaired the germination of corn seeds (Table 2), in both first and final readings.Association of the analyzed products showed no advantages to physiological quality and vigor in maize seed treatments. On average, association of all products causedan 8.9% decrease in germination at the first reading, and only 2.4%of the final germination, in relation to the control treatment. Association of products can promote antagonistic interaction effects between components, impacting the effectiveness of inputs as well as seed vigor.Results differ from those found in other studies. Several studies conducted to evaluate the interaction between seed treatment, fungicides, insecticides, fertilizers and inoculation of *Azospirillum brasilense*in maize seedshave reported no influence of inputs on the germination rate and several agronomical attributes in maize (Dartora *et al*., 2016; Mumbach *et al*., 2017).Demonstrating the importance of specific and product-oriented assessments considering different formulations and sources. Chemical elements, such as micronutrients, present in excess levels in the environment can affect the growth and metabolism of plant species (Marschner, 1995; Suganya, 2021) when the internal concentration of these elements exceeds the limit, it can cause a toxicity effect on plants. Toxicity of metals in plants can vary from species to species, and it is related to concentration, chemical structure, and soil pH (Ackova, 2018).Determining factors of the effects may occur depending on the source. In a study with zinc and boron applied to maize seeds, at the dose recommended by the manufacturer, there was no difference in germination between control and treated seed samples (Ribeiro *et al*., 1994).

Some authors have found that the addition of Mo in different doses before inoculation reduced the number of viable *Bradyrhizobium* cells in the seed, compared to the inoculation treatment without Mo, so this micronutrient applied to the seed may presented an antagonistic effect on the use of other products (Albino & Campo, 2001).Seed fertilization using micronutrients is usual but high dosages may affect seeds and their physiological quality (Pereira *et al*., 2012). The transgenic cultivar P30F53YH under Cold Test conditions, had, in general, a lower germination rate in comparison to the conventional cultivar P30F53 (Table 3). As observed with germination test, associations between tested products were also not advantageous, especially when liquid fertilizer was present.Better results were found when products were used in isolation, however with no differences in relation to control treatments. Ferreira*et al*. (2007)found that the emergence of maize seedlings under stress conditions (cold test), is affected in seed treatments when using the same liquid fertilizer assessed in this research, in the dosage of 10 mL kg^{-1} of seeds, causing a reduction in emergence in relation to control. Using a conventional hybrid (GNZ 2004) and a line (Le 57) under stress conditions,it was found thar biostimulant + micronutrientsassociation and the isolated micronutrient fertilizer use reduce the physiological quality of maize seeds (Silva *et al*., 2008). Results of this study corroborate those of the mentioned, assuming that there was a deleterious effect, or at least, disadvantageous for the quality of the maize seeds when treated with liquid fertilizers. Emergence Speed Index and emergence test results of the cultivar P30F53 YH presented a superior performance when compared to the conventional cultivar P30F53 (Table 5). These differences are possibly due to the characteristics of the seed lots used rather than genotypes, which differ only by the presence of a transgenic event. Initial seedling development measured by shoot dry weight (Table 3) was superior when the inoculant product was used in isolation and in association with the biostimulant.

Table 1. Analysis of variance summary for first reading (1st READ), final germination count (FGC), cold test (CT), root dry weight (RDW), shoot dry weight (SDW), emergence speed index (ESI) and emergence test (ET) of maize cultivars seedlings with seeds treated with different inputs

SV	DF	MEAN SQUARE						
		1st READ	FGC	CT	RDW	SDW	ESI	ET
Treat. (T)	7	89,2**	21,8*	306,0**	121382,5**	11117,4*	0,1	9,5
Cult. (C)	1	297,6**	5,0	2601,0**	1934025,5**	70456,7	4,1**	95,1**
TxC	7	14,8	8,5	139,6*	102046,3**	33803,6	<1	4,1
Error	48	9,4	6,0	37,0	17200,0	29050,8	<1	7,5
CV%		3,37	2,56	7,42	12,39	16,42	2,89	2,85

** ($P \leq 0,01$); * ($P \leq 0,05$)

Table 2. Germination percentage on first and final germination test readings of maize hybrid seeds treated with different products

Treatments	Germination (%)	
	1st Reading	Final
Control	95,0 a	96,3 a
Inoculant	93,5 a	96,3 a
Biostimulant	92,5 a	97,3 a
Micronutrients	92,8 a	97,0 a
Inoculant + Biostimulant	91,3 a	96,5 a
Inoculant + Micronutrients	87,8 b	93,2 b
Biostimulant + Micronutrients	86,5 b	93,3 b
Inoculant + Biostimulant + Micronutrients	86,5 b	94,0 b

Means followed by same letter do not differ significantly by Scott-Knott test.

Table 3. Percentage of germination in seedling cold test and shoot dry weight test of maize hybrids from seeds treated with different inputs

Treatments	Cold Test		Shoot Dry Weight (g)
	30F53	30F53YH	
Control treatment	88,0 aA	81,0 aA	10,2 b
Inoculant	93,0 aA	83,0 aB	12,9 a
Biostimulant	91,5 aA	88,0 aA	10,4 b
Liquid fertilizer	93,5 aA	65,5 bB	9,3 b
Inoculant + Biostimulant	90,0 aA	79,5 bB	11,9 a
Inoculant + Liquid Fertilizer	91,0 aA	72,5 bB	9,8 b
Biostimulant + Liquid Fertilizer	74,0 bA	68,5 bA	8,9 b
Inoculant + Biostimulant + Liquid Fertilizer	85,0 aA	66,5 bB	9,7 b
Mean	88,3 A	75,6 B	

Means followed by the same lowercase letter in the column, and means followed by the same uppercase letter in the row, do not differ significantly by Scott-Knott test ($\alpha = 5\%$)

Table 4. Dry root weight of seedlings (grams) of maize hybrids from seeds treated with different products

Treatments	30F53	30F53YH
Control	10,58 bA	8,59 aA
Inoculant	13,73 aA	7,30 aB
Biostimulant	14,04 aA	8,03 aB
Micronutrients	13,94 aA	7,43 aB
Inoculant + Biostimulant	14,74 aA	9,89 aB
Inoculant + micronutrients	12,47 aA	8,52 aB
Biostimulant + micronutrients	7,29 cA	8,42 aA
Inoculant + Biostimulant + micronutrients	13,92 aA	10,41aB
Mean	12,59 A	8,57 B

Means followed by the same lowercase letter in the column, and means followed by the same uppercase letter in the row, do not differ significantly by Scott-Knott test.

Table 5 . Emergence Speed Index (ESI) and percentage of germinated seedlings on emergence tests (ET) of maize cultivars 30F53 (conventional) and 30F53YH (transgenic), using different products for seed treatment

Cultivars	Tests	
	ESI	ET
30F53	8,75 b	95,06 b
30F53YH	9,23 a	97,50 a

Means followed by same letter do not differ significantly (Scott-Knott cluster analysis).

Other treatments, including control, were statistically lower. Increase inseedling dry weight from *Azospirilum* and biostimulant inoculated seeds is likely due to the production of growth-promoting substances, an effect proposed by both products. It is being reported phytohormones, mainly indole-acetic acid (IAA), excreted by *Azospirillum* play an essential role in promoting plant growth in general (Bashan *et al.,* 2004). Inoculating seeds with *A. brasilense* can promote an increase in seedling biomass during the initial stages of development, due to differential embryo development induced by the growth regulators produced by bacteria that penetrate the seed coat along with water, accelerating radicle growth and enhancing its absorption capability (Cassán *et al*., 2009;Cassán *et al*., 2016). Dry root weight in cultivar P30F53YH showed lower performance when compared with cultivar P30F53 for the tested treatments, with the exceptions of the control treatment and the Biostimulant + Micronutrients treatment (Table 4). Treatments with products applied in isolation and in association with the inoculant promoted an increase when compared to control, notably in cultivar 30F53. Treatment with biostimulant + micronutrients in association presented the lowest root development of maize seedlings, which should be cautionedthat its use requires greater attention.On the other hand, 30F53YH cultivar (Table 4) did not show any significant difference in dry root weight related to treatments, however it is observed that there was a tendence towards an increased dry root weight when seeds received treatments containing *Azospirillum* sp or biostimulant. Some studies have reported that the use of isolated *Azospirillum* showed the best performance in seedling root system development and shoot part characteristics in maize, in relation associated fungicides use (Vogel *et al*., 2019). This bacterium increasesthe biomass and root volume, especially in the initial stages of seedling development.Initial root system development makes crops more tolerant to water deficit and improve the ability to absorb soil nutrients due to a greater area of soil being able to be explored. These results are similar to those found by Silva *et al*. (2008) in maizein which the authors verified that seedling dry root weight, dry shoot weight, and total dry weight was reduced when the seeds received treatments with micronutrients and with the combination of biostimulant + micronutrients. In general, seed treatments with micronutrients associated with biostimulants interfered with the initial seedling development and caused physiological changes. Some products, when applied in isolation or in association with fungicides, may, in certain situations, cause a reduction in seed germination and seedling survival, due to phytotoxicity.

CONCLUSIONS

Biostimulant, micronutrient liquid fertilizer and *Azospirilum brasilense* inoculation used in isolation did not decrease germination levels of maize seeds lots. Combinations of biostimulant and *Azospirilum* promoted increase in physiological quality of seeds.

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