

Influence of bioregulator on physiological quality of maize seed during storage

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Abstract

Incorporation of vegetal regulator-based products to maize seeds is a new reality. However, special attention should be paid to the physiological quality of seeds, particularly during storage. The purpose of the present study was to assess the effects of the bioregulator Stimulate® about both seed treatment and maize seeds' physiological quality in different storage periods. Evaluation of physiological quality was accomplished by germination test (first and final count), cold germination test and sand emergence test. Maize seeds' bioregulator-managed treatment – especially when applied to high vigor seeds – ensures lower reduction in physiological quality for up to 180 days of storage, whereas, when applied to medium vigor seeds, greater bioregulator benefits are observed in short-term storage.

Keywords: *Zea mays*, bioregulator, germination, vigor

Influência de biorregulador na qualidade fisiológica de sementes de milho durante o armazenamento

Resumo

A incorporação de produtos a base de reguladores vegetais às sementes de milho é uma nova realidade. No entanto, deve-se atentar aos efeitos desses produtos na qualidade fisiológica das sementes, particularmente no armazenamento. O objetivo do trabalho foi avaliar os efeitos do biorregulador Stimulate®, via tratamento de sementes, na qualidade fisiológica das sementes de milho em diferentes períodos de armazenamento. A avaliação da qualidade fisiológica foi realizada pelos seguintes testes: primeira contagem e contagem final do teste de germinação, teste de frio e emergência em areia. Em sementes de milho de alto vigor o tratamento de sementes de milho com o biorregulador garante menor redução na qualidade fisiológica por até 180 dias de armazenamento. Em sementes de médio vigor, maiores benefícios do biorregulador são observados em períodos curtos de armazenamento.

Palavras-chave: *Zea mays*, regulador vegetal, germinação, vigor

Introduction

Seeds have become means of technology transfer for maize crop. Among these practices are the incorporation of several phytosanitary products, micronutrients and bioregulators. Techniques which induce greater germination and physiological quality are important factors to increase seed performance potential, and therefore the uniformity of plants under field conditions (Aragão et al., 2003).

According to Marcos Filho (2005), high quality seeds produce reliable results including strong, vigorous and well-developed plants which establish in different edaphoclimatic conditions with higher emergence speed, leading to a fast canopy closure of the row spacing, thus assuring a better development of the plants. On the other hand, the effects on either medium or low physiological quality are translated by the decrement on germination percentage; the increment on abnormal seedlings number and the reduction on seeds vigor, resulting in weak seedlings with little or no chance of establishing competitively in the field (Nakagawa, 1999). The depletion of both germinative power and vigor is the most marked manifestation of seeds' deterioration.

Seeds soaked in substrates containing growth-promoting substances consists of a widely known technique for years. Vegetal regulators are part of a group named bioregulators, formed by auxins, cytokinins and gibberellins, retarders, inhibitors and ethylene (Castro et al., 2008). In this present study, special attention will be given to the bioregulator Stimulate[®], which, according to Castro et al. (1998), is classified as a vegetal regulator containing a mixture of three phyto regulators and traces of minerals such as 0.005% indolebutyric acid (auxin); 0.009% kinetin (cytokinin) and 0.005% gibberellic acid (gibberilin). These regulators are defined as either natural or synthetic substances, which can be directly applied to plants, seeds or soil, in order to increase production and/or improve seed quality (Silva et al., 2008).

Factors such as the presence of hormones and the balance between them, promoters and inhibitors play an important role on the regulation of germinative process (Rodrigues, 2008). The

development of the embryonic axis is mediated by auxins and cytokinins, while the increment both in number and size of cells is due to the embryo protein synthesis. In general, gibberellins are involved in the transcription of gene coding; cytokinins in the translation and auxins in the membrane permeability. That gibberellic acid regulates the expression of α -amylase gene, which hydrolizes starch is responsible for the provision of reserves from the endosperm during seedling development.

Use of growth regulators on germination phase improves seedling performance by speeding up emergence and highlighting seed potential in a number of species such as beans (Alleoni et al., 2000); soybean (Ávila et al., 2008; Klahold et al., 2006; Moterle et al., 2008; Albrecht et al., 2010) cotton (Albrecht et al., 2009; Santos & Vieira, 2005; Vieira & Santos, 2005), forest essence (Prado Neto et al., 2007), sunflower (Santos et al., 2013) and lettuce seeds (Soares et al., 2012).

Nevertheless, the viability of this technology is still contradictory regarding maize seed treatments, besides lack of information related to storage. In addition, chemical treatment effectiveness depends on other factors such as seed vigor at the product application time.

Seed deterioration process is firstly expressed by both degradation and increased cell membrane permeability (Marcos Filho, 2005). Therefore, seeds may become more susceptible to environmental stress and sensitive to chemical application. In some occasions, the application of certain products might be harmful to seed quality, expressed mainly by the increased number of abnormal seedlings. However, according to Khan et al. (1978), the use of biologically active chemical compounds such as growth regulators may cease or diminish the impact of adverse effects on seed quality and performance.

Another issue to which attention should be given is the preservation of the quality of maize seeds during storage. Quality preservation until sowing time ensures a high potential of seedling emergence in the field, leading to productivity profits (Silva et al., 2008). Since seeds' treatment has been increasingly used and the level of seeds'

vigor may interfere with the action of regulators present in the Stimulate® of stored corn seeds, the objective of this present study was to assess the effects of the bioregulator Stimulate® about seed treatment on physiological quality of maize seeds in different periods of storage.

Material and methods

Experiment installation and conduction

This present study was carried out at the Universidade Estadual de Maringá (UEM), Maringá, Paraná State, Brazil. Evaluations on seed physiological quality were made at the Seed Technology Laboratory of the Núcleo de Pesquisa Aplicado à Agricultura (NUPAGRI) in the Agricultural Science Center of the Universidade Estadual de Maringá (UEM).

Maize seeds utilized for this experiment were the simple hybrids P30F53H and DKB240Y. Two seed lots from each hybrid were selected and framed within two quality levels (high and medium vigor). For both quality levels, seed samples whose germination test results were above or close to 85% and vigor indexes close to 90% (for high vigor) and 70% to 50% (for medium vigor) were chosen.

Hybrid seeds P30F53H were industrially treated with the fungicides Derosal Plus e Maxim XL in doses of 3 and 1.5 L t⁻¹ of seeds, respectively and with the insecticides K-Obiol and Actellic in doses of 0.08 and 0.016 L t⁻¹, respectively. For the hybrid DKB 240Y, industrial treatment contained the fungicide Captan in dose of 1.5 kg t⁻¹ of seeds and the insecticides K-Obiol and Actellic in doses of 0.06 and 0.04 L t⁻¹ of seeds, respectively. This treatment consists in industrial chemical treatment to control pests and diseases that are usually used for corn seed during storage.

Two trials represented by each hybrid were carried out. Seed lots of high and medium vigor for hybrids P30F53H and DKB 240Y were treated with Stimulate® in the dose of 12.5 mL kg⁻¹ of seeds and the witness received distilled water in the same volume of solution from that of the treatment with Stimulate®. In each treatment were used 2.5 kg of seeds and homogenization of the whole volume was made in 4 kg plastic bags. The set was agitated for 2 minutes in order to homogenize the mixture and then shadow

dried. Treated seeds were stored in room temperature for 0; 15; 30; 45; 60; 90; 120; 150 and 180 days at the Seed Technology Laboratory of the Núcleo de Pesquisa Aplicado à Agricultura (NUPAGRI) in the Agricultural Science Center of the Universidade Estadual de Maringá (UEM), Paraná State, Brazil.

Assessed features

The following tests were used in order to assess the physiological quality of maize seeds during storage periods:

Germination Test

This test was conducted by using 4 subsamples of 50 seeds for each lot, placed to germinate between three leaves germination paper (Germitest®) soaked with distilled water in the ratio of 2.5 times the dry paper mass. Rolls were made by wrapping up those paper towels and then taken to a germinator so that the temperature was maintained at 25°C (Brasil, 2009). Evaluations were accomplished at day four (first count) and day seven (final count), by taking into account the percentage of normal seedlings, in accordance to the criteria established in the Rules for Seed Analysis (Brasil, 2009). First count of normal seedlings was considered as vigor indicative and final count as the total percentage of seeds' germination.

Modified Cold Test

This test was conducted by using 4 subsamples of 50 seeds for each treatment. As subtract, three leaves of germination paper (Germitest®) soaked with distilled water were utilized. After sowing, rolls were made from those paper towels, wrapped by plastic bags and sealed with adhesive tape. The rolls were kept in this condition for seven days in a germination chamber so that the temperature was maintained at 10 °C. Then, rolls were taken to a germinator and kept at a constant temperature of 25 °C during four days and evaluation accomplished after this period (Barros et al., 1999). Results were expressed in percentage of normal seedlings on the assessment date, following the same criteria adopted by the germination test.

Final percentage of emergence

This test was carried out from sowing in trays containing 8 kg of washed and sterilized sand with 4 replications of 50 seeds for each treatment. Seedlings emerged were then counted 15 days after sowing, according to Nakagawa (1999).

Experimental design and statistical analysis

Both experiments represented by the hybrids P30F53H and DKB 240Y were arranged in split-plot in a randomized complete block design with four replicates. Treatments were distributed in a factorial scheme 2 x 2, the first factor is the seed treatment (with and without the bioregulator Stimulate®) and the second one is the level of vigor (high and medium vigor). For subplots, the

periods of storage (0; 15; 30; 45; 60; 90; 120; 150 and 180 days) were evaluated.

Variables characterizing physiological quality of seeds were all submitted to variance analysis by using SISVAR statistical analysis system. Regression analysis was used to verify polynomial model adjustment for dependent variables in relation to storage periods at 5% probability level, according to Banzatto & Kronka (2008).

Results and discussion

First count of germination test

Regression models and determination coefficients resulting from the regression analysis for the percentage of normal seedlings on the first count germination day, evaluated in the hybrids P30F53H and DKB 240Y, are shown in Figure 1.

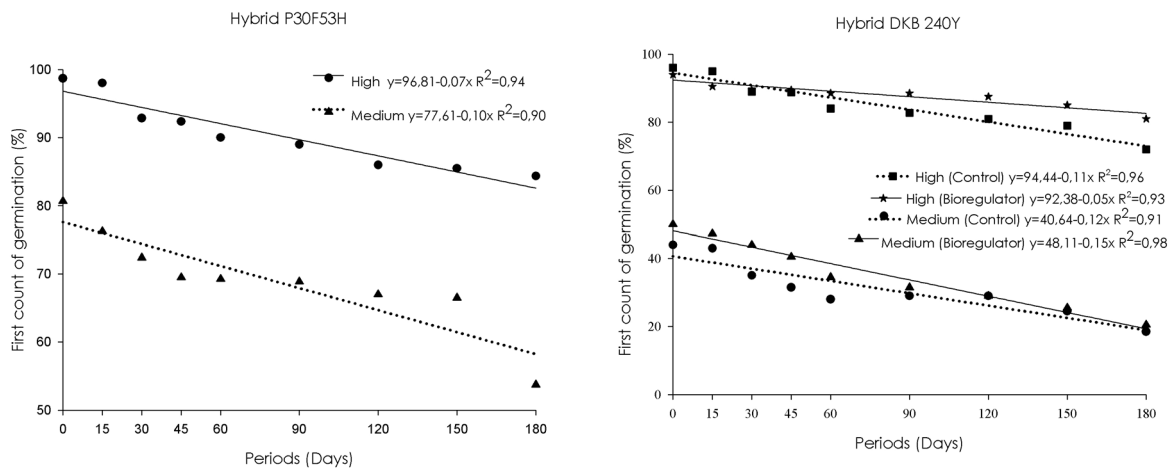


Figure 1. Polynomial regression for normal seedling percentage on the first count of germination test in high and medium vigor maize seed lots for hybrids P30F53H and DKB 240Y during storage periods. Maringá, Paraná State, Brazil

For hybrid P30F53H (Figure 1), vigor results on the first count are related to the development of the interaction period x vigor, because all other interactions – first and second order – did not present significant effect ($P>0.05$). According to the results, there were no significant reductions in this variable in both vigor levels during the period of storage evaluated. Medium and high vigor levels presented reductions of 0.10% and 0.07% in the percentage of normal seedlings for each day of seed storage, respectively. According to Goneli et al. (2005), vigor level in maize seeds directly influences in the reduction intensity for this characteristic during storage, leading to the conclusion that deterioration process is enhanced by storage.

In hybrid DKB 240Y (Figure 1), for high vigor level, treatment with the bioregulator promoted seed vigor loss for each storage day (0.05%). Vigor reduction was markedly observed in the witness, 0.11% for each day in which seeds were stored. Thus, treatment with the bioregulator, particularly for high vigor lot provided lesser impact on the reduction of vigor for maize seeds during storage. These results corroborate with those found by Ferreira et al. (2007), in which greater vigor of maize seeds were also verified with the bioregulator Stimulate® treatment and subsequent 6-month period storage. These authors observed a greater α -amylase enzyme activity in maize seeds treated with Stimulate® and further storage for 6 months.

Bioregulator Stimulate® is comprised by balanced quantities of the main hormone groups, thus, its action on the hormonal system triggers "de novo" synthesis of α -amylase, which may lead to a greater enzymatic activity. At the beginning of hydration, hydrolytic enzymes, such as amylases, proteinases and α -glucanases become activated in the embryo. In cereal, amylase activity is essential to provide both energy and carbon skeletons for the development of the embryo through usable products produced by cellular respiration breaks. α and β -amylase enzymes are involved in the main degradation system of seed starch. This alteration on enzyme activity ratifies lower vigor reductions observed in this present study during storage of seeds treated with the bioregulator for the high vigor lot.

For hybrid DKB 240Y (Figure 1) – medium vigor lot – seed treatment using the bioregulator presented some reduction (0.15%) for each storage day, while for the witness, this reduction was 0.12%. Thus, treatments using the bioregulator showed bigger tendency on vigor reduction over

180-day storage. Therefore, there is evidence that for not so long storage periods, bioregulators are able to maintain its initial performance for medium vigor maize seeds.

Germination Assessment

Results on germination test (Figure 2) for hybrid P30F53H indicated percentages above 90 for high vigor lot throughout storage period, providing compliance to the referenced standards of maize seeds trading (Brasil, 2005). Nevertheless, the witness – high vigor lot – presented linear-adjustment equation with significant reduction of 0.04% in germination percentage for each storage day. For maize seeds treated with the bioregulator, average germination percentage throughout storage period was 97.43%. In a study carried out by Ferreira et al. (2007), no differences on seed germination for the simple hybrid seeds GNZ 2004 and for L57 lineage were observed when seeds were treated with the bioregulator Stimulate® (15 mL kg⁻¹ of seeds).

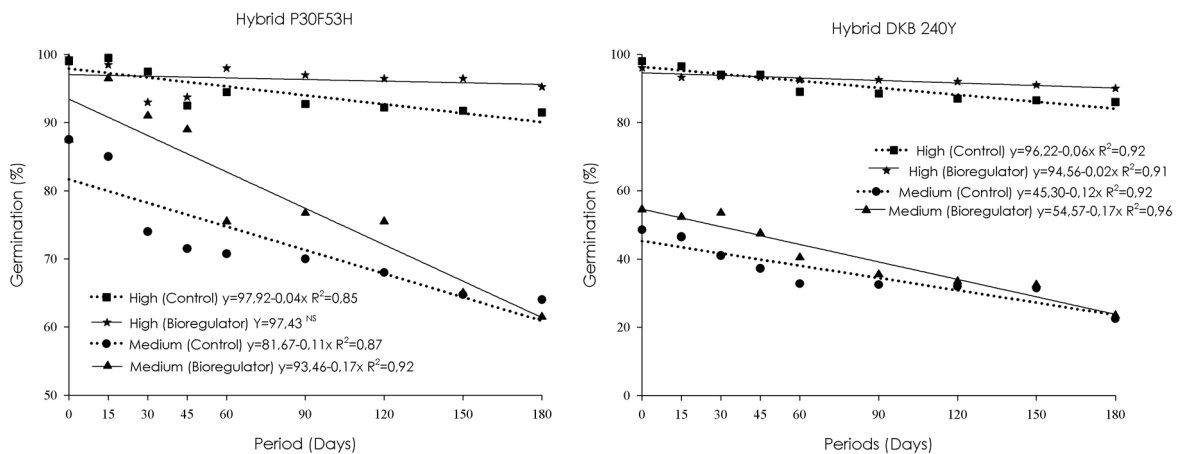


Figure 2. Polynomial regression for normal seedlings percentage on the germination test in lots of high and medium maize seeds vigor for hybrids P30F53H and DKB 240Y during storage periods. Maringá, Paraná State, Brazil

For hybrid DKB 240Y, in high vigor seeds (Figure 2), the percentage reduction of normal seedlings on the germination test for each storage day was 0.02% and 0.06%, in treatments with the bioregulator and the witness, respectively. These results demonstrate the potential of the bioregulator in promoting a smaller reduction in germination percentage of high vigor maize seeds during storage. Soares et al. (2012) claim the pre-soaking of lettuce seeds in plant growth regulator, under favorable conditions, even

without changing their germination capacity improves the speed of germination and seedling vigor, increasing the chances of successful crop establishment.

In medium-vigor seed lots (for both hybrids), there was a significant reduction in germination percentage during storage (Figure 2) in both witness and the treatment with the bioregulator. However, the decrease of germination percentage in treatments with the bioregulator for each storage day was 0.17% for

hybrid P30F53H and 0.15% for hybrid DKB 240Y. In the witnesses, reductions were in the order of 0.11% and 0.12% for each storage day for hybrids P30F53H and DKB 240Y, respectively.

Results obtained by other authors (Alleoni et al., 2000; Ávila et al., 2008; Klahold et al., 2006; Moterle et al., 2008; Santos & Vieira, 2005; Silva et al., 2008; Albrecht et al., 2009) with the same product in different crops, attested that there is evidence that the bioregulator Stimulate® must alter the physiological potential of seeds.

Bioregulator is composed of gibberellic acid, which, among other functions, affects protein metabolism that may increase the rates of enzyme synthesis involved in seed germination process (Taiz & Zeiger, 2009). Findings by Aragão et al. (2003) confirms that GA₃ (gibberellin) induces reserve degradation enzyme synthesis due to the increase of α-amylase activity, once a "de novo" synthesis of this enzyme occurs during

germination. In addition, it has been established that GA₃ induced an increase of other amylase activities. Therefore, elevated degradation rates of reserve proteins associated with high α-amylase activity provided an increase on germination and vigor of supersweet maize seeds treated with GA₃ (50 mgL⁻¹). According to Dissanayake et al. (2010), germination of guayule (*Parthenium argentatum* Gray) seeds is regulated by the balance between promoters and inhibitors. These authors still state that either endogenous or exogenous growth regulators can alter the balance to inhibit or induce germination.

Vigor Assessment in the modified cold test

For vigor assessment determined by cold test (Figure 3), there were significant reductions in normal seedling percentage in both vigor levels and seed treatments during storage.

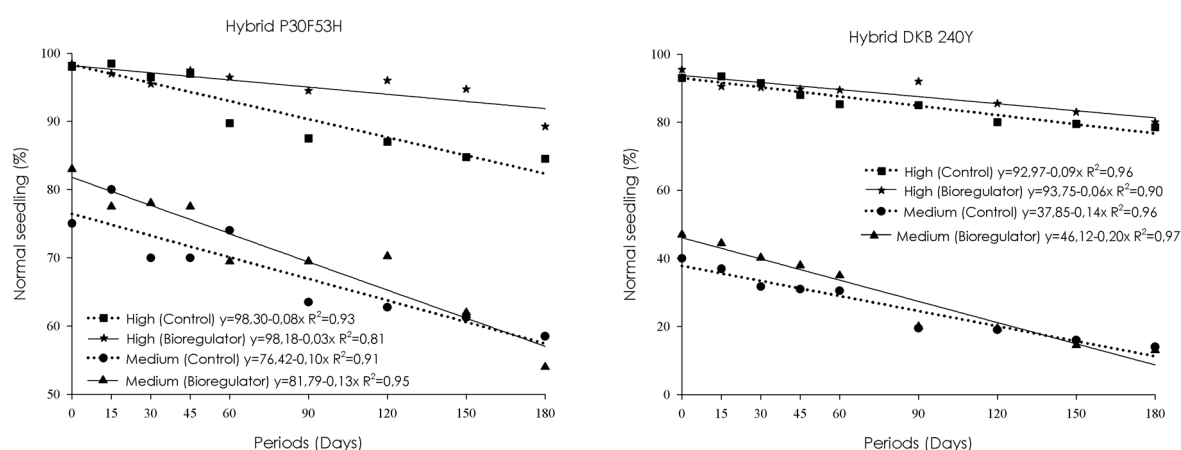


Figure 3. Polynomial regression for normal seedling percentage in the cold test in high and medium vigor maize seeds for hybrids P30F53H and DKB 240Y during storage periods. Maringá, Paraná State, Brazil

For each storage day, vigor reductions were observed in high vigor maize seed lots, treated with the bioregulator in the order of 0.03% and 0.06% for hybrids P30F53H and DKB 240Y, respectively (Figure 3). In high vigor seeds (witnesses), reductions reached 0.08% and 0.09% for each storage day for hybrids P30F53H and DKB 240Y, respectively. Results obtained by Castro & Vieira (2001) shown that Stimulate® application on maize seed treatment (10 mL kg⁻¹) was efficient in improving seed performance, providing a greater number of normal seedlings. According to Leszczynski et al. (2012) the application of the bioregulator did not improve the percentage of

seed germination and no positive response of the seedlings was reported from the onion cultivars 'Baia Periforme' and 'Bola Precoce'. However, seedling vigor of 'Híbrida Superex' was positively affected by the application of the bioregulator.

This result characterizes the positive effect of the bioregulator treatment in high vigor maize seeds to vigor maintenance during storage period. They also prove that there is a bioregulator action on seed hormonal system, because α-amylase is synthesized by gibberellin action, leading to a greater genetic expression and enzyme production, which is intrinsically related to greater seed vigor (Marcos Filho, 2005).

The presence of hormones and the balance between each other, i.e., promoters and inhibitors play a crucial role in vegetal regulator-guided monitoring (Taiz & Zeiger, 2009); thus, the development of the deterioration process observed during storage might happen by altering hormonal balance in maize seeds, which, in turn, benefits the bioregulator action on promoting better performance with the increase of storage period.

In medium vigor seed lots, treatment with the bioregulator presented a decrease in vigor levels for each storage day of 0.13% for hybrid P30F53H and 0.20% for DKB 240Y (Figure 3). Witnesses presented reduction percentages in maize seed vigor in the order of 0.10% and 0.14% for each storage day for hybrids P30F53H and DKB 240Y, respectively.

Silva et al. (2008), in a study on maize seeds' treatment with the bioregulator Stimulate®, observed that, under stress conditions, there was a decrease in the physiological quality of seeds. The authors report lower peroxidase activity in seeds treated with this bioregulator, consequently, providing a higher sensitivity to the effects of O₂ and free radicals, what may have influenced on seed germination and vigor values which were submitted to such treatment.

Final Emergence Assessment

In the final emergence assessment accomplished 15 days after sowing for both hybrids (Figure 4) as well as for other variables evaluated, there was a decrease in this variable due to storage development of maize seeds, independent of initial vigor level and seed treatment.

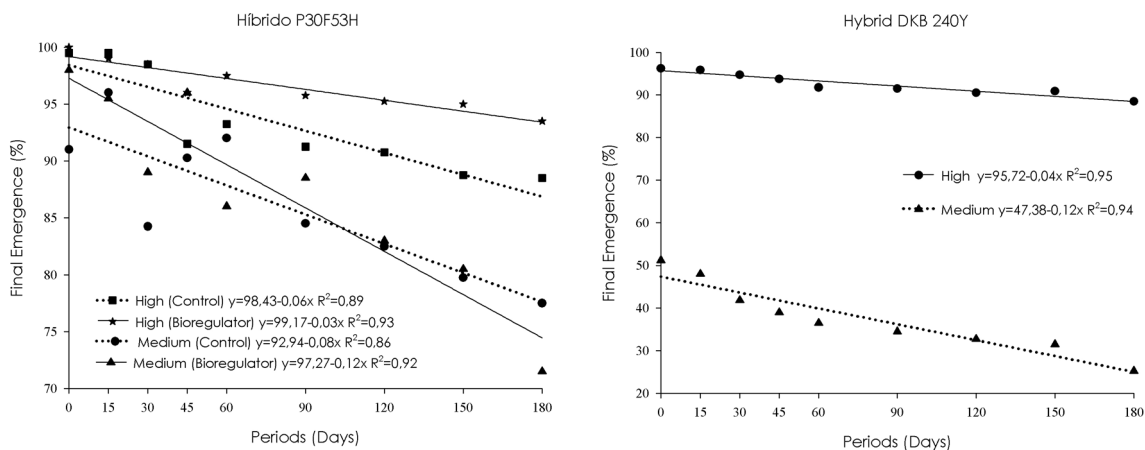


Figure 4. Polynomial regression of final emergence percentage in sand 15 days after sowing of high and medium vigor seed lots for hybrids P30F53H and DKB 240Y during storage periods. Maringá, Paraná State, Brazil

For hybrid P30F53H (Figure 4), in the high vigor lot, special attention is given to the treatment with the bioregulator whose decrease in final emergence variable for each storage day was 0.03%. While for the witness, this decrease reached 0.06%.

Santos et al. (2013) concluded the pre-soaking of sunflower seeds with plant growth regulator Stimulate® (4 mL Stimulate® L⁻¹ in pre-soak for 4 hours) increased germination, promoting the formation of more vigorous seedlings and reducing the percentage of seedlings abnormal, and promote higher percentage of seedling emergence. Similarly, Santos & Vieira (2005), while working with Stimulate® application on

cotton seeds (7.0; 14.0; 21.0; 28.0; 35.0 and 42.0 mL kg⁻¹ of seeds) obtained greater percentage of emergence in both sand and vegetal earth proportional to the product dose increment. However, Ferreira et al. (2007) did not observe any significant effect on maize seed emergence from Stimulate® treatment in the dose of 15 mL kg⁻¹ of seeds.

Results on medium vigor seeds for hybrid P30F53H (Figure 4) have indicated that treatment with the bioregulator presented greater reduction in final emergence percentage throughout storage. In this treatment, reductions were 0.12 for each storage day when compared to the witness (0.08%).

Due to these results, it is mentioned that in the beginning of the storage period, seeds might be at the first stages of deterioration process and, thus, the bioregulator is efficient enough to promote benefits to seed physiological performance. Castro & Vieira (2001) verified that bioregulator application to seeds proved to be efficient on germinative process performance, providing greater number of normal seedlings and significantly reducing seedling abnormalities.

For Krzyzanowski & França Neto (2001), deterioration has an inverse relationship to vigor, i.e., the higher seed deterioration, the less vigor and vice-versa. They also highlight that physiological changes are easily characterized, for instance, the emergence percentage reduction, seedling slow growth and abnormal seedling production.

Results on final emergence percentage for hybrid DKB 240Y (Figure 4) refer to the development of storage period in relation to seed vigor. Analysis results in high vigor seeds reveal lower intensity in percentage reduction of final emergence for each storage day (0.04%) while in medium vigor seeds, vigor was 0.12%.

Greater development of deterioration processes in medium vigor seeds might happen because maize seed treatment with the bioregulator has not provided positive influence for final seedling emergence during storage.

Conclusion

Throughout the storage period of maize seeds, there has been a linear reduction on physiological quality for hybrids P30F53H and DKB 240Y for both initial vigor levels and seed treatment. In high vigor maize seed lots, the reduction in physiological quality during 180-day storage are less intense in treatments with the bioregulator. In medium vigor seeds, greater reduction on seed physiological quality has been noticed in long-term storage. □

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