Temperature, potassium nitrate, substrate, and harvesting time on the germination of zoysia grass seeds

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Abstract

Zoysia japonica grass has commercial importance to Brazil due to its use in golf courses and football stadiums. The main concern about its commercial cultivation is the unevenness on seed germination as well as the ideal seed harvesting time. Therefore, the objective was to measure the effect of temperature and potassium nitrate on dormancy overcoming, and of substrate and harvesting time on germination of zoysia grass seeds (Zoysia japonica Steud). There were three experiments: 1) temperature and potassium nitrate treatments, with six temperature conditions (constants at 20 °C, 25 °C, 30 °C, and 35 °C, and alternating at 20-30 °C and 20-35 °C), under absence or presence of potassium nitrate; 2) temperature and substrate treatments, with two temperature conditions (alternating at 20-30 °C and 20-35 °C) and three types of substrates (on paper, between paper, and on sand); and 3) harvesting time treatments, which seeds were obtained by manual harvesting comprising 11 harvesting periods. Seeds germinated more rapidly on the sand and paper, at the alternating temperature of 20-35 °C. The best period for seed harvesting was from 18 to 19 days after ear emergence in the production field.

Keywords: Zoysia japonica, lawn, dormancy, physiological maturation

Introduction

The lawn is one of the most important components in landscape composition. In addition to its aesthetic value, it works by stabilizing the soil, preventing erosions caused by wind and water, intercepting rainfall, reducing the formation of mud and dust in residences caused by traffic and, also, improving water infiltration in the soil (Saifuddin and Osman, 2014). Furthermore, it helps in lowering the air temperature, reduces clearings in forests, and serves as a recreation area.
Currently, most ornamental grasses are commercially propagated asexually, however, due to some advantages of sexual propagation, seed trade has grown in the worldwide market, including Brazil, that still shows great prospects for internal sale. In fact, the use of seeds for the formation of new lawns is a common practice in Europe and in the United States and is currently considerably expanding also in Brazil (Batista et al., 2015).

Some species are propagated almost exclusively vegetatively, mainly due to difficulties of obtaining good quality seeds. Furthermore, Gallagher and Wagenius (2016) mention that overall plug survival of some grass species is at least three times greater than that of directly sown seedlings. Although having good initial development, the grass formed from vegetative material presents several agricultural limitations, such as general high costs, major need of manpower, greater chance of spreading pests and diseases, and need of large amounts of propagating material for extensive areas. Therefore, the use of vegetative material may then be both logistically and economically impracticable (Gallagher and Wagenius, 2016), so seed sowing should be financially preferable (Stevens et al., 2015).

In general, there is little information in the literature on seed technology of ornamental and sporting grasses. The Brazilian Rules for Seed Analysis (Brasil, 2009) provide recommendations on temperature, substrate, and dormancy overcoming generalized for zoysia grass; however, there are no other references in the Brazilian literature recommending these indications and, currently, with several varieties available in the market, it is not known if those recommendations are valid for all; similarly, little is known about the harvesting time effect on the germination of grass seeds. For Z. japonica seeds, Brasil (2009) also recommends the use of potassium nitrate (KNO₃) at the germination time for dormancy overcoming.

The objective was to evaluate the effect of temperature, potassium nitrate, substrate, and harvesting time on the germination of zoysia grass seeds (Zoysia japonica).

Material and Methods

The study was carried out at a laboratory with two lots of Z. japonica seeds. The first batch, characterized as Z. japonica ‘Zenith’, was commercially available, containing the following information in the package: 99.72% purity; 85% germination; pretreatment with sodium hydroxide (without concentration specification); 0.27% inert matter; 0.01% weed seeds; and 0% dormancy. The second batch, characterized as Z. japonica ‘Esmeralda’, was obtained by manual harvesting at Itograss Agrícola Alta Mogiana Ltda., located in Sales Oliveira city, in São Paulo State, Brazil.

For all experiments, the following germination percentage tests and determinations, evaluated at 35 days after sowing, were performed using four replicates of 100 seeds; germination rate was assessed along the germination test, and it was evaluated daily from the seventh day after sowing, which calculation was determined according to the equation proposed by Maguire (1962); and the water content was determined by drying the seeds under the temperature of 105 ± 2 °C after 24 hours (Brasil, 2009), with four replicates of 200 seeds. The criteria used to evaluate the germination test were based on recommendations described in the Rules for Seed Analysis (Brasil, 2009), considering the germinated seeds that originated normal seedlings with all essential structures.

The effect of temperature and potassium nitrate on seed germination of Z. japonica ‘Zenith’ was carried out using only seeds obtained from the commercial lot. The statistical design was completely randomized, in a 6 x 2 factorial scheme, with six temperatures (constant at 20, 25, 30, and 35 °C, and alternating at 20-30 and 20-35 °C) and presence or absence of potassium nitrate. For the treatment with potassium nitrate, seeds were placed on filter paper saturated with a 0.2% solution of potassium nitrate (KNO₃) (2 g KNO₃ dissolved in 1 L water), according to Brasil (2009).

Commercial seeds of Z. japonica ‘Zenith’ were also used to study the effect of temperature and substrate on germination. The statistical design was completely randomized, in a 2 x 3 factorial scheme, with two alternating temperatures (20-30 and 20-35 °C) and three substrates (on paper, between paper, and on sand) with four replicates of 100 seeds.

For the evaluation of the harvesting periods, seeds of Z. japonica ‘Esmeralda’, comprising the second batch, were used. The statistical design was completely randomized with 11 treatments and four replicates of 100 seeds. Each treatment was represented by a seed harvesting period, so 11 samples were collected, that is, 11 treatments. Seeds were harvested at the beginning of ear emergence up to 36 days, i.e., 0, 4, 8, 11, 15, 18, 22, 25, 29, 32, and 36 days after ear emergence, when natural degranulation occurred.

Sowing was performed on filter paper and the germination test was maintained at the alternating temperature of 20-35 °C. Seeds were collected from plants cultivated under common irrigation and maintenance pruning conditions (approximately 5 cm high). This area was 24 m x 25 m and was subdivided into 60 plots of 10 m² (2 m x 5 m), which were randomly numbered from 1 to 15, with four replicates, representing the harvesting periods. The criterion to determine collection time was based on Toledo et al. (1981) for Bahia grass seeds (Paspalum notatum Flüggé).

Data were statistically analyzed and averages compared by the Tukey test at 5% probability of error. For the study of the harvesting period effect, unfolding of degrees of freedom was performed to verify the variable behavior as a function of the seed harvesting periods. Germination percentage data were transformed into arc sine (x/100)½ for statistical analysis.

Results and Discussion

The interaction between temperature and potassium nitrate was significant for both germination percentage and germination rate (Table 1), what proves that seed dormancy was overcome. When the germination percentage of both treated and untreated seeds is compared, temperatures of 20, 25, and 30 °C presented higher values when seeds were treated with potassium nitrate and, at the other temperatures, no significant differences were observed.
The alternating temperatures promoted higher germination percentage when seeds were treated with KNO₃, while lower temperatures (20 and 25 °C) resulted in germination under 50%, also the lowest. Among the tested temperatures for untreated seeds, results showed the same trend, so the alternating temperatures were the best for germination when potassium nitrate was not used.

Comparing the temperatures for both treated and untreated seeds, the temperatures that provided highest germination percentage were 20-30 °C and 20-35 °C, which did not differ statistically from 30 °C for treated seeds. Thus, regardless the treatment with potassium nitrate, the alternating temperatures at 20-30 and 20-35 °C were those that promoted greater germination (Table 1).

Table 1. Germination percentage and germination rate (GR) of Zoysia japonica ‘Zenith’ seeds submitted to different temperature conditions, treated or untreated with potassium nitrate.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Germination (%)</th>
<th>Potassium nitrate</th>
<th>GR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>20 °C</td>
<td>30 Ac</td>
<td>3 Bd</td>
<td>1.7524 Ac</td>
</tr>
<tr>
<td>25 °C</td>
<td>49 Abc</td>
<td>28 Bc</td>
<td>4.3125 Ab</td>
</tr>
<tr>
<td>30 °C</td>
<td>68 Aab</td>
<td>51 Bbc</td>
<td>7.2119 Aa</td>
</tr>
<tr>
<td>35 °C</td>
<td>51 Abc</td>
<td>61 Ab</td>
<td>3.9917 Bb</td>
</tr>
<tr>
<td>20-30 °C</td>
<td>84 Aa</td>
<td>87 Aa</td>
<td>6.5143 Aa</td>
</tr>
<tr>
<td>20-35 °C</td>
<td>83 Aa</td>
<td>88 Aa</td>
<td>9.6767 Aa</td>
</tr>
<tr>
<td>CV (%)</td>
<td>13.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same upper-case letters in the line and lower-case letters in the column do not differ from each other by the Tukey test at 5% probability of error.

At the temperatures of 20 °C and 30 °C, the germination rate was significantly higher when seeds were treated with potassium nitrate, however, there were no significant differences between treated and untreated seeds at the temperatures of 25 °C and 20-30 °C; nevertheless, for the temperatures of 35 °C and 20-35 °C, the untreated seeds presented superior results.

The highest germination rates for treated seeds were obtained from the treatments 30 °C, 20-30 °C, and 20-35 °C; for untreated seeds, best results were obtained at the alternating temperature of 20-35 °C, which did not statistically differ from 20-30 °C.

The results about the temperature, for both germination percentage and germination rate, are in agreement with the Rules for Seed Analysis (Brasil, 2009) since it does indicate, for germination of Z. japonica seeds, treatment with potassium nitrate, moistening the substrate initially with a 0.2% solution at the temperature of 20-35 °C. The alternating temperature of 20-30 °C for both treated and untreated seeds may also be indicated, as well as 30 °C for treated seeds.

This seems to happen for other grass species too, as Fakhfakh et al. (2018) also found that germination percentage of Stipagrostis ciliata (Desf.) De Winter untreated seeds was higher at 25 °C, presenting good results as well at 25-30 °C. However, high temperatures of 35 °C or above inhibited germination, what is probably due to decreasing water absorption combined to a low gas diffusion (Fakhfakh et al., 2018).

Analyzing the germination percentage and considering the alternating temperatures of 20-30 °C and 20-35 °C that presented the best results, it was observed that the potassium nitrate was enough to break dormancy, as there were no differences among treated and untreated seeds. However, for the germination rate, it was noted that the treatment with potassium nitrate, when added to the sodium hydroxide, was harmful. For Brachiaria decumbens Stapf, for instance, Tomaz et al. (2015) found that either sulfuric acid or nitrate potassium may be disregarded if seeds are sown under the alternating temperatures of 20-35 °C, what could even mean that germination procedures would be faster, easier, and less costly.

Potassium nitrate is also used to overcome seed dormancy for other grasses, such as Brachiaria brizantha (Hochst. ex A. Rich.) Stapf ‘Piatã’ (Lima et al., 2015). However, B. brizantha ‘Marandu’ behaved differently and did not have its dormancy overcome, suggesting that the cultivar Piatã presents chemical dormancy (Lima et al., 2015). Carvalho and Nakagawa (2012) report that, in most cases, potassium nitrate is efficient to overcome seed dormancy; for grass seeds, dormancy would be due to the occurrence of nitrogen-fixing substances (phenolic compounds) in the skin-pericarp complex. However, Penado et al. (2018) observed changes in germination after rice (Oryza sativa L.) seeds endured an air plasma treatment, with decreased number of trichomes on seed surface that probably contributed to minor surface roughness and major water absorption. Therefore, some grass species may also present physical dormancy.
Table 2. Germination percentage and germination rate (GR) of *Zoysia japonica* ‘Zenith’ seeds submitted to different temperature conditions and substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Germination (%)</th>
<th>GR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-30 °C</td>
<td>20-35 °C</td>
</tr>
<tr>
<td>On sand</td>
<td>82 Aab</td>
<td>83 Aa</td>
</tr>
<tr>
<td>On paper</td>
<td>87 Aa</td>
<td>88 Aa</td>
</tr>
<tr>
<td>Between paper</td>
<td>79 Bb</td>
<td>89 Aa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Germination (%)</th>
<th>GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-35 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.15</td>
<td>13.20</td>
</tr>
</tbody>
</table>

Means followed by the same upper-case letters in the line and lower-case letters in the column do not differ from each other by the Tukey test at 5% probability of error.

Table 2 presents the results of the variance analysis for the effect of temperature and substrate on seed germination of *Z. japonica* ‘Zenith’. The interaction between factors was significant for germination percentage, but, for germination rate, only the independent factors were significant (Table 2).

For the alternating temperature of 20-30 °C, germination percentage was significantly higher when seeds were sown on paper, which did not differ significantly from those that were placed on sand; for the alternating temperature of 20-35 °C, there was no difference among the substrates.

There was statistical difference between the alternating temperatures only for the substrate between paper, so the alternating temperature of 20-35 °C presented the best result. For the other substrates, there was no significant difference between both temperatures tested.

Regarding the germination rate, seeds sown on paper germinated faster, but did not differ statistically from those placed on sand. The temperature that provided the highest rate was 20-35 °C.

These results are in agreement with those substrates recommended in the Rules for Seed Analysis (Brasil, 2009), which are on paper, between sand, and on sand; however, the substrate between sand was not tested due to the difficulty of visualizing the seeds under these conditions because of seed size and color.

For the harvesting times, there was a quadratic regression adjustment for both germination percentage and rate, as higher values occurred at 18.4 and 18.6 days after ear emergence in the field of production for, respectively, germination percentage and germination rate (Figure 1).

**Figure 1.** Germination Percentage (A) and Germination Rate (B) of *Zoysia japonica* ‘Esmeralda’ seeds according to different seed harvesting periods (0, 4, 8, 11, 15, 18, 22, 25, 29, 32, and 36 days after ear emergence).

The physiological maturity is the moment when dry matter transfer from the mother plant to the seeds is stopped, presenting then the highest physiological quality and vigor (Carvalho and Nakagawa, 2012). Therefore, maximum germination (Figure 1A) indicates that seeds were at the physiological maturity point, also confirmed by the germination rate (Figure 1B), resulting in high-vigor seeds at that stage.

There was a reduction in germination percentage from harvesting after 22 days of ear emergence (Figure 1A), probably due to seed exposure to adverse environmental conditions, such as temperature, and risk of pest and disease...
attack, compromising physiological quality. However, the most appropriate seed harvesting time varies according to the species. Smiderle et al. (2008), for instance, when working with rice 'BRS Jaburu', verified that the most favorable seed harvesting time fell between 29 and 36 days after flowering.

The water contents obtained at ear emergence (0 day) were 13%; four days afterwards was 17%; 8 days, 22%; 11 days, 20%; 15 days, 22%; 18 days, 20%; 22 days, 22%; 25 days, 21%; 29 days, 13%; 32 days, 12%; and 36 days, 15%. Higher water content, closer to ear emergence, is due to transport processes of assimilates to the seeds that are accumulated during the maturation process (Bewley et al., 2013), as it may also be observed for the Z. japonica 'Esmeralda' seeds. This highest physiological quality of Z. japonica 'Esmeralda' seeds was obtained with high water content, as it was also found in rice seeds, cultivar BRS Jaburu (Smiderle et al., 2008).

The water content was much higher at 4, 8, 11, 15, 18, 22, and 25 days after ear emergence when compared to the water content of seeds obtained in the market (12%). This is probably due to seed drying before storing, as such high water content may accelerate the deterioration process.

Even for those harvesting periods that presented best results, germination percentages were very low, i.e., 30%, 32%, and 30%, respectively, for 15, 18, and 22 days. At these dates, water levels were very high, that is, 22%, 20%, and 22%, respectively, for 15, 18, and 22 days after ear emergence. Maximum germination of 34.5% would be obtained at 18.4 days after ear emergence. However, according to Moreira et al. (2014), drier seeds indicate that more solutes, besides water, were leached, which also influences germination negatively. Furthermore, the permanence of high water contents, allied to environmental conditions, may have contributed to seed deterioration and reduction of seed vigor (Figure 1B). Indeed, Bareke (2018) reports that, when hygroscopic equilibrium is reached, the seed moisture content still may change according to variations in relative humidity.

In fact, according to the climatic data of the region, it was verified that, along the seed harvesting periods, there was a total precipitation of 175 mm, with average temperatures of 23.0 °C and 23.8 °C, confirming the variation and high water content of the seeds from 22 days after ear emergence. From the maximum germination, occurring at 18.4 days after ear emergence, there was a reduction in germination (Figure 1A), what may be due to a deterioration process reducing seed physiological quality as well as vigor (Figure 1B).

Dudle (1996) suggests a storage period for grass seeds after harvesting, so that seeds may germinate to their full potential. Also, Toledo et al. (1981) suggest that P. notatum seeds should be stored for 14 months in dry chamber for complete loss of dormancy. Similarly, Amaro et al. (2015) report that dormancy of Crambe abyssinica Hochst. ex R.E. Fr. seeds may be partially overcome from six months of storage. In this study, seeds were stored for six to seven months, however, a longer storage period in a dry chamber could have been enough to decrease water content and break dormancy of Z. japonica 'Esmeralda' seeds, just as it was verified for P. notatum. Dudle (1996) stated that the storage period may vary according to species and varieties. High water content and lack of processing or treatment to overcome dormancy in seeds certainly interfere negatively in seed germination as, according to Moreira et al. (2014), grass seeds do have different dormancy causes, so different methods to overcome it should be used.

In the packing specifications of the seeds purchased in the market, germination percentage was 85%. In this study, it was observed that, at 10 months after the initial analysis, the percentage reached 88% (seeds germinated at the alternating temperature of 20-35 °C under the absence of potassium nitrate).

**Conclusions**

Temperatures providing highest germination percentage and faster germination rate of Zoysia japonica 'Zenith' seeds were the alternating 20-30 °C and 20-35 °C. Substrates providing higher germination percentage of Zoysia japonica 'Zenith' seeds were on paper and on sand at the alternating temperature of 20-30 °C, and on paper, between paper, and on sand at the alternating temperature of 20-35 °C. Seeds germinated faster when placed on paper and on sand under the alternating temperature of 20-35 °C.

The potassium nitrate is efficient on overcoming dormancy of Zoysia japonica 'Zenith' seeds. The best period for harvesting Zoysia japonica 'Esmeralda' seeds are from 18 to 19 days after ear emergence in the field of production for promoting higher germination percentage and germination rate.

**Author Contribution**

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References


