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Heat treatment to overcome seeds dormancy of *Panicum maximum* cultivars (Poaceae)

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The guinea grass *Panicum maximum*, an African grass, is one of the main forage grasses in tropical America. Its propagation is mainly carried out by seeds, but seed dormancy hampers good pasture establishment. The aim of this study was to evaluate the use of heat treatment to overcome seed dormancy of *P. maximum* cultivars Milênio, Tanzânia and Mombaça. Seeds of each cultivar have been subjected to heat treatments at temperatures of 50, 60 and 70°C with exposure time for 5, 10 and 15 h. Afterwards, they were placed in germination test. The data were subjected to analysis of variance and, when significant, polynomial regression was performed, with up to 5% of probability. The variables analyzed were germination percentage, germination speed index (GSI) and average germination time (AGT). *P. maximum* seeds of different cultivars showed distinct responses to heat treatment. The cultivar Tanzânia responds positively to different combinations of temperature and periods of seeds exposure, and the treatment at 70°C for about 8 h is recommended to overcome dormancy with better germination performance. For cultivar Milênio, it is recommended the exposure of seeds at 70°C for 15 h. Cultivar Mombaça is negatively influenced by the heat treatment, so this treatment is not recommended.

Key words: African grasses, germination, pasture, temperature.

INTRODUCTION

The guinea grass (*Panicum maximum* Jacq.), originating in Africa, is one of the main forage grasses in tropical America, with great importance in pasture establishment. It presents good range of adaptation to tropical and subtropical conditions, high forage production of good quality, good resistance to grazing, being well accepted by animals. Its propagation is mainly carried out by seeds (Dias and Alves, 2008). The use of quality seeds with high seed germination is essential to a good pasture establishment, with high technological level (Chiodini and Cruz-Silva, 2013). However, *P. maximum*, and most forage species have low seed germination, which may be

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related to the presence of seed dormancy (Martins and Silva, 2003).

Dormant seeds are those which, although viable, do not germinate in a specific period of time under any combination of normal physical environmental factors (temperature, light/dark, etc.) which, on the other hand, is favorable to its germination, that is, after the seed becomes non-dormant (Baskin and Baskin, 2004). This mechanism is one of the main strategies of plant species to increase their survival rates and seedling establishment, because it allows germination to occur only when it is more likely that the conditions for seedling establishment are appropriate (Finch-Savage and Leubner-Metzger, 2006). However, dormancy affects emergence speed of plants in the field, resulting in an irregular stand, which slows pasture establishment and favors the appearance of invasive plants (Martins and Silva, 2003; Almeida and Silva, 2004; Lacerda et al., 2010).

Dormancy expression in tropical grasses is associated with physiological causes (present in freshly harvested seeds and suppressed during storage) or physical causes (related to restrictions imposed by the seed coat to oxygen and water uptake by seeds) (Whiteman and Mendra, 1982). However, the current state of knowledge is unsafe to indicate procedure settings that are capable of preventing the expression of dormancy on pasture establishment. Several studies have shown the efficiency of the chemical treatment use, with immersion of seeds in sulfuric acid for different time periods, in order to overcome seed dormancy of forage grasses species such as *P. maximum* (Smith, 1979; Martins and Silva, 1998), *Brachiaria brizantha* (Garcia and Cicero, 1992; Montório et al., 1997; Lago and Martins, 1998; Munhoz et al., 2009), and *B. dyctioneura* (Almeida and Silva, 2004). However, this method presents technical and operational risks to the environment (Almeida and Silva, 2004; Martins and Silva, 2006) and it is of difficult adaptation to seeds which, because of structural features, are easily damaged by the process (Martins and Silva, 1998). Thus, considering risk reduction, heat treatment to overcome dormancy has been presented as an alternative.

Heat treatment can be applied via moist heat (hot water or steam) or dry heat. This last one has a lower heat capacity and heat exchange than the wet one, requiring therefore longer exposure time (Lazarotto et al., 2013). However, further studies are needed to better define the binomial temperature/time for each species for the use of the dry heat as a method to overcome seed dormancy (Alencar et al., 2009). This study evaluated the use of heat treatment to overcome seeds dormancy of *P. maximum* cultivars Tanzania, Mombaça and Milênio, preserving their germination and vigor.

MATERIALS AND METHODS

This study was carried out at Embrapa Beef Cattle, Campo Grande(MS), Brazil. Three *P. maximum* cultivar seeds have been used (Tanzânia, Mombaça and Milênio), just after harvest. After harvesting and processing, the seeds of each cultivar were placed in beakers and subjected to heat treatments at temperatures of 50, 60 and 70°C, with time exposure for 5, 10 and 15 h in a kiln with forced air circulation. The zero time, without application of heat, was considered as control. For each treatment combination (temperature and exposure time) 1.0 g of seed of each cultivar was used. Just after the heat treatment, the seeds were put to germinate.

A completely randomized design was used. For each treatment (combination of temperature and time exposure), four repetitions of 100 seeds were used, put in plastic boxes (gerbox) with germitest paper, previously moistened with distilled water (2.5 times the weight of germitest paper). The seeds were then incubated in germination chamber Biochemical Oxygen Demand (BOD) with photoperiod of 8 hours and alternated temperatures of 15/35°C during 28 days, according to the Seed Analysis Rules (Brazil, 2009). Evaluations of germinated seeds were carried out daily. The variables evaluated were:

Germination (%): Considering as germinated seeds the ones which presented at least 2.0 mm of seminal root (Juntila, 1976).

Average germination time – AGT (days): Estimated by the formula of Krzyzanowski et al. (1999): AGT = $(\Sigma n_i t_i)/\Sigma ni$, where: n = number of germinated seeds per day and t_i = incubation time (days).

Germination speed index – GSI: determined according to the formula of Maguire (1962), GSI = $\Sigma(n/t)$, where: n= number of germinated seeds in the computed first, second, …, and last count; t= number of days from sowing to first, second, …, and last count.

At the final count tests, the seeds remaining in the substrate and not germinated were tightened with a pincer. The ones with the seed coat softened, which broke with the pressure were considered dead.

The data (germination percentage, germination speed index and average germination time) were subjected to analysis of variance and, when significant, polynomial regression was performed (Pimentel Gomes, 2009), with up to 5% of probability. Germination percentage data were transformed into arcsine $\sqrt{x/100}$ (Sokal and Rohlf, 1995).

RESULTS

Heat treatment influenced the seed germination of the three *P. maximum* cultivars evaluated. For cultivar Tanzânia, all evaluated temperatures showed quadratic adjustments between the exposure times to treatment and the variables analyzed. Except to 70°C/15 h treatment, all other treatments enhanced germination of this cultivar as it provided an increase in the percentage of germination, AGT reduction and increased GSI compared to control (Figure 1). The higher germination percentage (54.4%) was obtained in the treatment at 70°C/7.56 h, representing an increase of almost nine times the germination obtained in the control (6.25%). Treatments with temperatures 50 and 60°C showed similar germination maximum point (49.6%), and, thus, lower percentage than in treatment at 70°C (Figure 1A). However, these two treatments (50 and 60°C) showed better results for vigor than 70°C treatment, once they had lower AGTs (Figure 1B) and higher GSIs (Figure 1C).

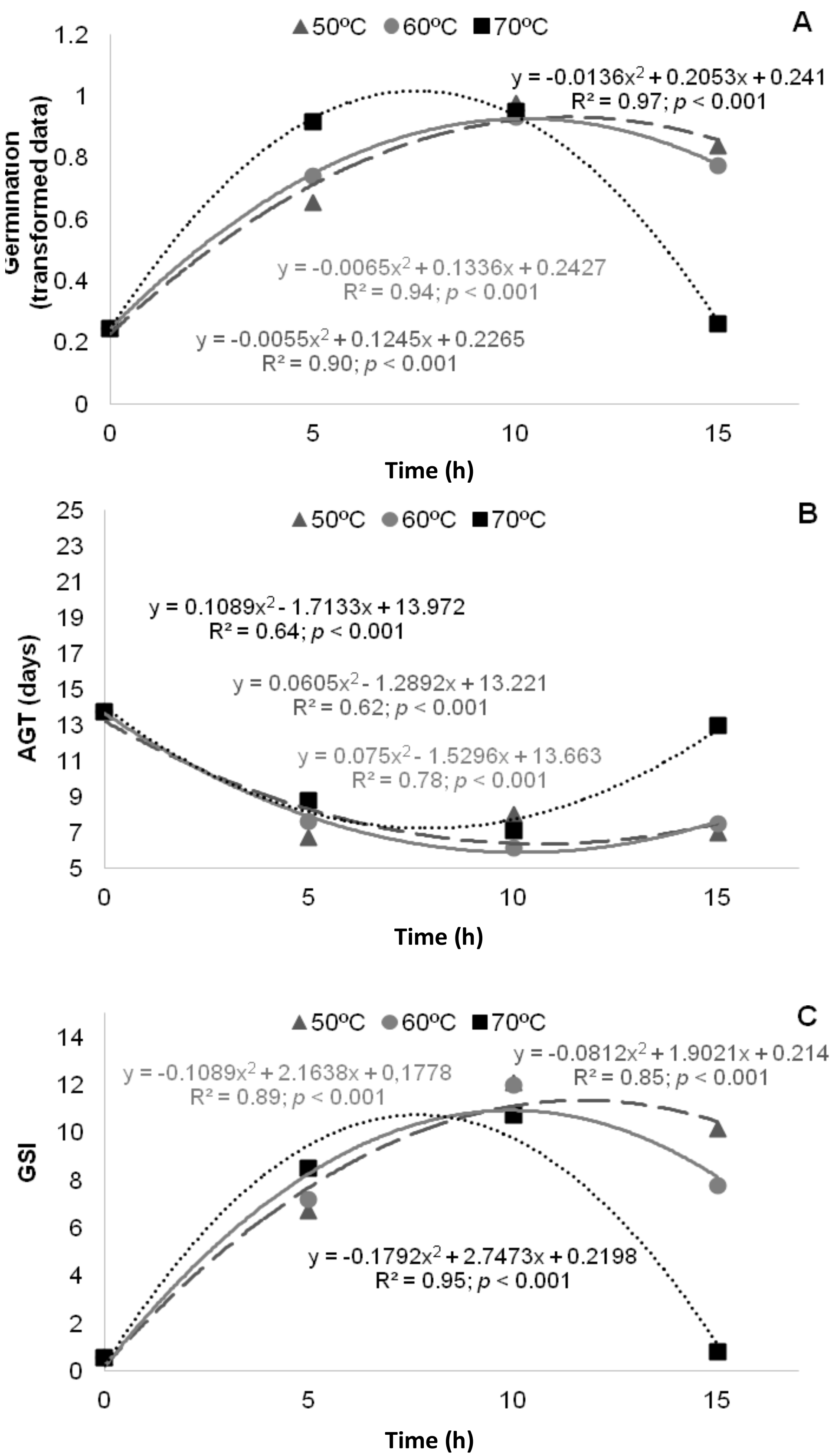

Figure 1. Germination (A), Average germination time (B) and Germination speed index (C) of *P. maximum* seeds cultivar. Tanzânia treated with temperatures (50, 60 and 70°C), by different exposure times (0, 5, 10 and 15 h).

For Milênio cultivar, only the treatment at 70°C resulted in response of all variables (Figure 2). At this temperature, a linear increase of germination percentage was obtained with increasing treatment time, and the exposure of the seeds for 15 h at this temperature resulted in an increase of about 3.2 times in germination (Figure 2A). Furthermore, treatment at 70°C/15 h resulted also in maximal reduction AGT (Figure 2B) and increased GSI (Figure 2C), indicating that this treatment positively influenced the seed vigor. Although the treatment at 50°C has shown a tendency to reduce AGT with increasing exposure time of seeds (Figure 2B), the low regression adjustment found ($R^2 = 0.30$), combined with the lack of response of this temperature for the other variables suggest the ineffectiveness of this treatment to overcome seed dormancy. Treatment at 60°C was not effective in overcoming seed dormancy of Milênio cultivar either (Figure 2).

The Mombaça cultivar, in general, was negatively affected by the heat treatment for overcoming seeds dormancy (Figure 3), resulting in reduced germination (to 50 and 60°C) (Figure 3A), increase in AGT (Figure 3B) and reduction of GSI for all temperatures evaluated (Figure 3C). Despite the fact that treatment at 70°C has resulted in a linear increase of germination in relation to the exposure time (Figure 3A), the detrimental effect in terms of seed vigor (increase of AGT and reduced GSI) suggests that it should not be used.

DISCUSSION

The study of alternatives to overcome fodder seed dormancy can contribute to the development of methods capable of industrial application, enabling their commercialization with dormancy partial or totally eliminated (Martins and Silva, 2003). The most indicated method would be the one which requires less time and economic investment, coupled with its easy handling in laboratory routines. Since dormancy overcoming treatments should simulate the environmental conditions in which the seeds are subjected in their natural habitat, tropical species tend to respond well to methods which use heat exposure (Garcia and Baseggio, 1999). Specifically in the case of grasses such as *P*. *maximum*, the seeds after releasing the parent plant are located predominantly on the soil surface, being exposed to soil action and fire (natural or artificial). Thus, these species have been selected as expression of survival and adaptation mechanisms at high temperatures (Martins and Silva, 1998).

In one of the few studies reported in the literature about the application of heat treatment in *P. maximum* seeds, Martins and Silva (1998) submitted seeds of the species to temperatures of 40, 55, 70 and 85°C for 5, 10 and 15 h. The results showed that exposure of the seeds to 40°C for 5 and 15 h were more favorable treatments because they resulted in overcoming seed dormancy and caused less damage during storage. Higher temperatures, despite being able to reduce the percentage of dormant seeds had latent deleterious effects on the storage.

The decrease in *P. maximum* seed dormancy rate during storage is a characteristic variable with the lot, harvest season and the genotype (Smith, 1979; Harty et al., 1983; Condé and Garcia, 1985). If this variation among genotypes of the species is expected in the natural seeds dormancy overcoming, it may be possible that it can be also found when using artificial treatments to overcome it. The study of Martins and Silva (1998) makes no reference to which cultivar was used and the present study found significant variation in the response to heat treatment between different cultivars of *P. maximum*. Cultivars Tanzania and Milênio had better germination response (percentage and speed) to 70°C temperature, although there are differences in exposure time of seeds suitable for each cultivar (Figures 1 and 3). On the other hand, Mombaça cultivar responded negatively to the tested temperatures and, in this case, it is possible that the use of temperatures below the tested in the present study may represent a strategy for overcoming dormancy of this cultivar, as found by Martins and Silva (1998).

There is little information available in the literature about the mechanisms that regulate the physiological response of seeds when exposed to high temperature. One possibility is related to the synthesis of proteins. Di Nola et al. (1990) found that exposure of *Echinochloa crus-galli* seeds for up to two hours at 46°C after soaking for 96 h at 36°C stimulated seed germination, affecting the composition of soluble proteins linked to cell membranes during the transition from the dormant stage to non-dormant. Furthermore, Martins et al. (1998) found that the glumes of *P. maximum* seeds submitted to thermal treatments showed shrinkage of their cells (stress resulting from the application of heat), which could interfere with seed permeability to gases and water. Thus, treatments that promote physical disintegration of the pericarp, eliminating their vulnerability, could be agents of overcoming seed dormancy.

In addition to dormancy, the incidence of pathogens in fodder species can negatively influence the establishment of pastures. This second factor promotes the reduction of productivity and quality, as well as a significant impediment to exports due to phytosanitary barriers (Marchi et al., 2008; Mallmann et al., 2013). High incidence of pathogen levels can reduce the viability of seeds or even promote their death even before germination (Mallmann et al., 2013). Some studies have shown that heat treatments can be an alternative for the control of pathogens (Muniz, 2001; Oliveira et al., 2011; Gama et al., 2014). However, this treatment is a measure that causes physiological and biochemical changes in the seeds at different intensities and may compromise their performance (Coutinho et al., 2007). Thus, the initial application of heat to the seeds for the purpose of determination of the temperature and heat

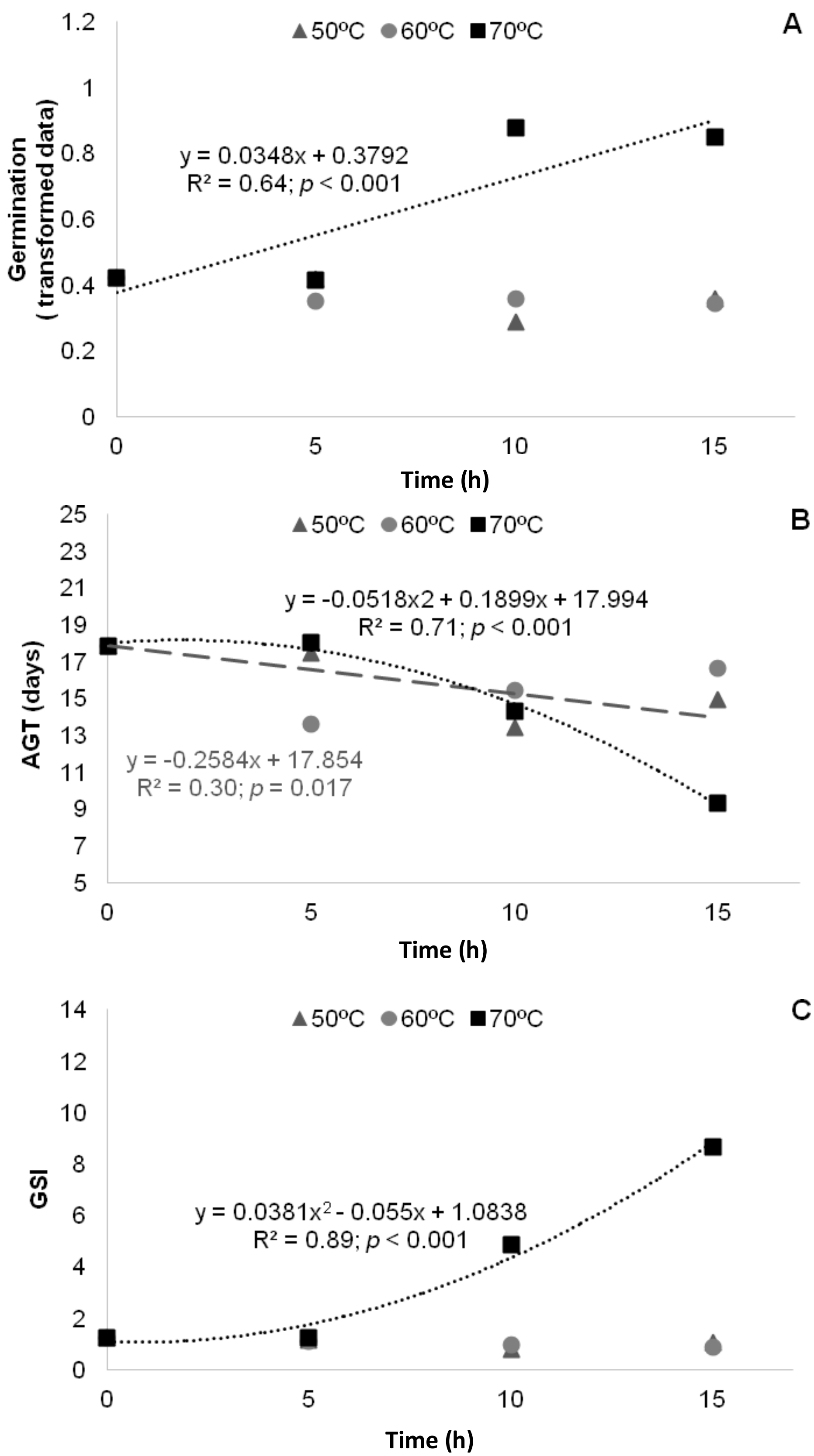

Figure 2. Germination (A), Average germination time (B) and Germination speed index (C) of *P. maximum* seeds cultivar. Milênio treated with temperatures (50, 60 and 70°C), by different exposure times (0, 5, 10 and 15 h).

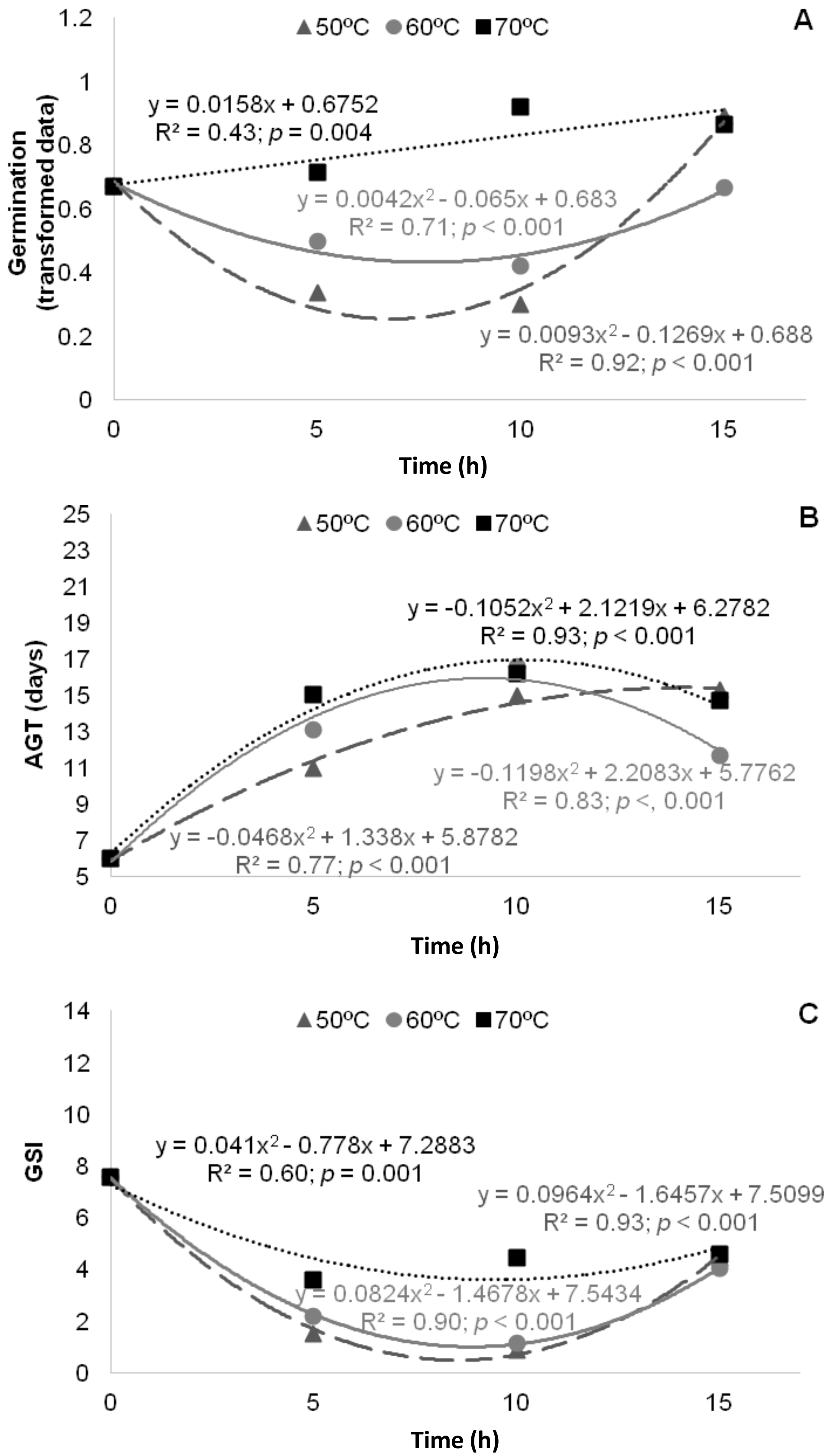

Figure 3. Germination (A), Average germination time (B) and Germination speed index (C) of *P. maximum* seeds cultivar Mombaça treated with temperatures (50, 60 and 70°C), by different exposure times (0, 5, 10 and 15 h).

application time in different species and varieties which do not reduce germination may represent a starting point for the phytosanitary control.

Conclusion

P. maximum seeds of different cultivars have distinct responses to heat treatment. The cultivar Tanzânia responds positively to different combinations of temperature and period of seeds of exposure, and the treatment at 70°C for about eight hours is recommended to overcome dormancy with better germination performance. For cultivar Milênio, it is recommended the exposure of seeds to 70°C for 15 h. Cultivar Mombaça is negatively influenced by the heat treatment, so this treatment is not recommended for this cultivar. Thus, treatments to overcome dormancy of seeds should also take into consideration the specificity of different cultivars of a particular species.

Conflict of interest

The authors have not declared any conflict of interest.

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