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DORMANCY BREAKING AND GERMINATION REQUIREMENTS FOR SEEDS OF *SPHAEROPHYSA KOTSCHYANA* BOISS.

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Abstract

A clear understanding of germination requirements and dormancy breaking methods of species has a direct impact on the success of the programs for the conservation of rare and endemic species. *Sphaerophysa kotschyana* is a halophytic endemic species naturally growing in the vicinity of Salt Lake. Due to the seed coat having hard, thick and water impermeable, the germination rates of the seeds are very low. This is a significant problem for the survival of the species. In this study, it was investigated that the effects of different methods on the germination of *S.kotschyana* seeds including acid (soaking in H_2SO_4, HNO_3 and HCl for 15, 30, 60, 90 and 180 minutes), hot water (soaking in dIH_2O at 50, 70 and 90 ˚C for 5, 10, 15 and 30 minutes) and mechanical scarification with sandpaper. The fastest and most effective seed germination was obtained by exposing to H_2SO_4 and thinning of the coat by sandpapering. When compared to the control, HCl and HNO_3 treatments for 180 minutes increased germination and germination rates were determined as 17.33% and 26.67% respectively. It was observed that hot water treatments generally encouraged germination and the highest germination observed in the seeds soaked for 10 minutes at 90 ˚C (22.67%). Based on the results of the study, it can be suggested that H_2SO_4 treatments and sandpapering were the most effective methods in germination of *S.kotschyana* seeds.

Key Words: Dormancy, hot water, physical dormancy, scarification, *Sphaerophysa kotschyana*

Introduction

Seed germination is one of the most important stages in life cycles of plants. Germination is affected by all of the environmental factors affecting vegetative growth. Germination requires favourable temperature, oxygen, water and lack of inhibitory substances in the environment. Seeds of many plant species cannot germinate despite favourable environmental conditions required for germination. Main reasons for this problem, which is termed as seed dormancy, are hard and impermeable seed coat and presence of immature or dormant embryo [1]. Seed dormancy is categorized as physical, physiologic, morphologic, morpho-physiologic and combined dormancies [2]. Physical dormancy is one of the most common types of dormancies among plant species. Physical dormancy is caused by water-impermeable seed coat (or fruit) [2] and is known to be observed in 16 families of angiosperms [3]. Leguminosae family is one the families in which this dormancy type is most common. Due to hard and water-impermeable seed coat in many species of this family, water and oxygen penetration inside the seed is inhibited and thus seed germination is delayed or inhibited [4]. Techniques such as mechanical scarification with sandpaper [5, 6, 7], chemical scarification with acids (H_2SO_4, HNO_3 and HCl) [1, 6, 8, 9], soaking of seeds in hot water for a short time [1, 8, 10] and perforation of seed coat [11, 12, 13, 14] are the most commonly used methods in breaking physical dormancy in seeds.

The genus *Sphaerophysa* DC. is distributed in Middle and Central Asia, from South Siberia to North China, West Caucasia and Anatolia. This genus is represented two species in the world. These species are *S. kotschyana* Boiss. and *S. salsula* DC. *S. kotschyana*, is a perennial, salt-tolerant endemic species which is distributed in the vicinity of Salt Lake (Tuz Golu), Konya in Central Anatolia and included in NT (Near Threatened) IUCN category [15]. Although no medicinal uses were identified for this species, due to its developed root system, this species is among the species which can be used for combating erosion. *S. kotschyana*, which has flashy flowers, is threatened due to reasons such as climatic changes and prolonged drought. Low germination rate due to hard and water impermeable seed coat has a negative impact on the survival of this species. One of the most important steps in programs for the conservation of *S. kotschyana* is to identify germination requirements of the seeds.

As far as our literature survey could ascertain, there was no study on the seed germination of this species. This study aims to investigate the effects of different treatments (i. mechanical scarification with sandpaper, ii. chemical scarification with H_2SO_4, HNO_3 and HCl and iii. soaking in hot water) in breaking dormancy of *S.*

kotschyana seeds and to determine the most effective method for germination.

Materials and Methods

Seed Collection

Seeds of *S. kotschyana* were collected in July 2010 from around Yavsan Saltworks (Salt Lake), Konya (lat. 42°90'336" long. 36°51'649" at an altitude of 910 m), Turkey. After collection, immature seeds and those attacked by insects were removed and the healthy seeds were stored at 4°C.

Evaluation of Seed Viability

Viability of 4 replicates of 25 *S. kotschyana* seeds were assessed using the tetrazolium chloride (TTC) staining technique [16]. Seeds were bisected along the longitudinal axes to evaluate the reaction of seed tissue to 2,3,5-triphenyltetrazolium chloride after incubation at 30 °C, in the dark for 2 h. Sections were observed on a stereoscope under fluorescent light and viable seeds with embryo and endosperm red stained, were counted.

Determining Physical Dormancy in *S. kotschyana* Seeds

A total of 50 mature and equal-sized seeds were divided into two groups of 25 and first weights of the seeds were recorded. The seeds in the first group were directly transferred to petri dishes containing dIH_2O, while the seeds in the second group were placed in petri dishes containing dIH_2O after scarification of seed coats with sandpaper. The seeds were let to germinate in an incubator with a 12-hour photoperiod at 24 °C for 5 days. At the end of this period, weights of the seeds were measured and number of germinated seeds was recorded.

Germination Experiments

Non-treated seeds were used as control. Mature and equal-sized seeds were divided into groups of 25. The following treatments were applied to break physical dormancy in *S. kotschyana* seeds.

Mechanical scarification with sandpaper

Seed coats were scarified by hand with sandpaper for 1 minute. Later the seeds were washed with dIH_2O.

Chemical scarification

The seeds were soaked in H_2SO_4 (98%), HNO_3 (63%) and HCl (37%) for 15, 30, 60, 90, 120 and 180 minutes. The seeds were then washed with dIH_2O.

Soaking in hot water

The seeds were transferred to beaker containing dIH_2O at 50, 70 and 90°C and were kept at hotplate for 5, 10, 15, 30 minutes. At the end of this period, the seeds were cooled, transferred to petri dishes and were let to germinate.

After each treatment, surface sterilization of the seeds was performed by keeping the seeds in 1% sodium hypochlorite for 5 minutes and washing thoroughly with dIH_2O. The seeds were later transferred to petri dishes containing double-layer Wathman No.1 filter paper soaked in 7 ml dIH_2O and were let to germinate. Three replicates of 25 seeds each were used for each treatment. Prepared petri dishes were placed in incubator with 70% moisture and 12-hour photoperiod at 24 °C and were let to germinate for 30 days. Seeds were considered to be germinated with the emergence of the radicle [17, 18]. Germinated seeds were counted every other day.

Germination percentages, germination rate indexes (GI), mean germination times (MGT) and germination rates (GR) of the seeds were calculated at the end of the experiment. MGT was calculated using Ellis and Robert's equation [19] to assess the GR.

$$MGT = \frac{\Sigma D_i.N_i}{N} \qquad GR = \frac{1}{M_{GT}}$$

where, MGT is the mean germination time, N_i the number of seeds germinated on the day i, D_i the days of germination test, N the total number of seeds, and GR is the germination rate.

The index of germination rate (GI) was estimated by using a modified Timson's index of germination velocity.

$$\mathrm{GI} = \frac{\sum G}{t}$$

where G is percentage of seed germination at 2-d intervals and t is total germination period (30 days) [20]. The maximum value possible using this index with our data was 50 (i.e., 1500/30). The higher the value, the more rapid the rate of germination.

Statistical Analysis

All treatments were carried out on a completely randomized design. All data obtained were subjected to one-way analyses of variance (ANOVA) and the mean differences were compared by Duncan test. Each data point was the mean of three replicates (n = 3) and comparisons with P values < 0.05 were considered significantly different. In all the figures, the spread of values is shown using error bars representing standard errors (SE) of the means.

Results

Evaluation of Seed Viability and Physical Dormancy

Tetrazolium test showed that *S. kotschyana* seeds which are 3.5-4 mm long, in reniform shape and dark brown colour have 98% viability.

It was observed that none of the seeds in the first group germinated and weights remained unchanged at the end of 5-day incubation period. On the other hand, it was determined that all of the seeds in the second group germinated starting from day 2 and it was found that *S. kotschyana* seeds had physical dormancy.

Effects of Dormancy Breaking Methods on Seed Germination

Maximum germination rate in non-treated *S. kotschyana* seeds was 4%. It was found that dormancy breaking treatments significantly affected germination rate.

Mechanical scarification with sandpaper

Germination rate was 100% in *S. kotschyana* seeds which underwent scarification of seed coat by sandpaper (Table 1). It was found that sandpapered seeds had the highest germination rate index (GI) (49.79). Mean germination times (MGT) of seeds which underwent mechanical scarification with sandpaper and germination rates (GR) were calculated as 2.16 and 0.46 respectively (Table 1).

Chemical scarification

HCl treatments

Germination in the seeds treated with HCl showed a significant increase when compared to the control (Fig. 1). The highest germination was found to be 17.33% in the seeds soaked in HCl for 180 minutes.

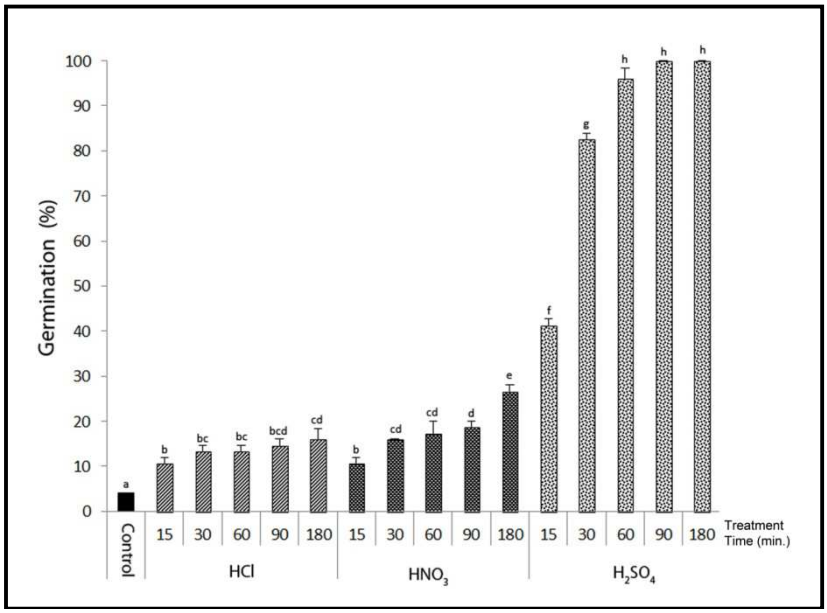

Fig. 1. The effects of different acid treatments (HCl, HNO_3 and H_2SO_4) on the germination of *S. kotschyana* seeds. Vertical bars indicate ± standard error (SE). Different letters are statistically significant at the P < 0.05 level as analysed by Duncan test.

The highest GI and GR and the lowest MGT values were determined in the seeds soaked in HCl for 180 minutes (Table 1; Fig. 2).

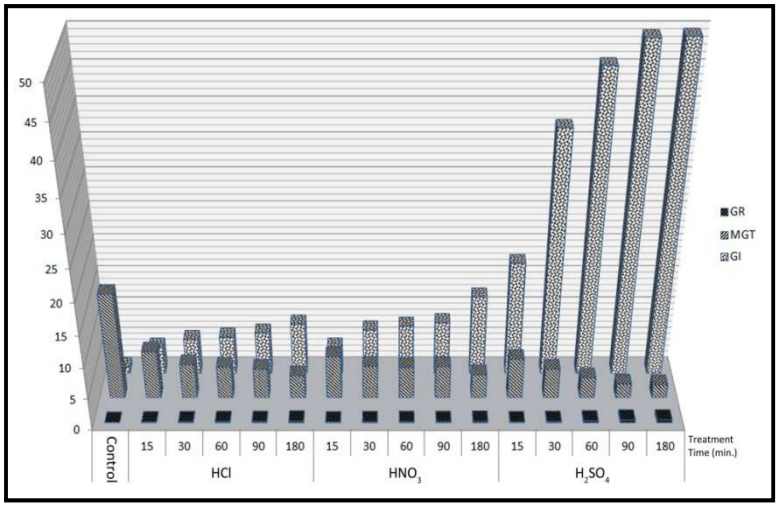

Fig. 2. The effects of different acid treatments (HCl, HNO_3 and H_2SO_4) on GI, GR and MGT values of *S. kotschyana* seeds ($p < 0.05$).

HNO_3 treatments

The highest germination (17.33%), GI (8.13) and GR (0.28) values in chemical scarification treatments with HNO_3 and the lowest MGT value (3.63) were determined in the seeds soaked in HNO_3 for 180 minutes (Fig. 1-2). It was found that the most effective HNO_3 treatment was soaking the seeds for 180 minutes.

H_2SO_4 treatments

It can be stated that the most effective chemical scarification method in breaking seed dormancies of *S. kotschyana* is H_2SO_4 treatments. Germination percentage, GI and GR values at the end of all H_2SO_4 treatments were found to be quite high, while MGT value was quite low when compared to the control group. It was found that the most effective treatment time was 180 minutes among H_2SO_4 treatments. Germination percentage, GI, GR and MGT values in this time were 100%, 49.78, 2.13 and 0.47 respectively (Fig. 1-2; Table 1).

Hot water scarification

Hot water scarification techniques have a significant positive impact on breaking dormancies of *S. kotschyana* seeds. It was found that germination increased due to the increase in soaking times at 50 °C and 70 °C. The highest germination increase was observed in 30-minute time in both treatments (Figure 3). Hot water treatment at 90 °C on the seeds for 5 and 10 minutes significantly increased germination percentages when compared to the control. On the other hand, soaking of the seeds in hot water at 90 °C for 15 and 30 minutes significantly negatively affected germination rate (Fig. 3).

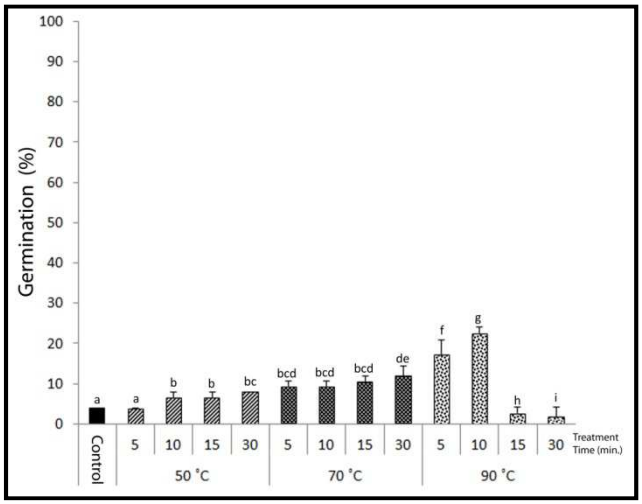

Fig. 3. The effects of different hot water treatments (50 °C, 70 °C and 90 °C) on the germination of *S. kotschyana* seeds. Vertical bars indicate ± standard error (SE). Different letters are statistically significant at the $P < 0.05$ level as analysed by Duncan test.

The highest germination, GI and GR values and the lowest MGT value in hot water treatments were found to be 22.67%, 10.89, 0.32 and 3.17 in the seeds soaked for 10 minutes (Fig. 4).

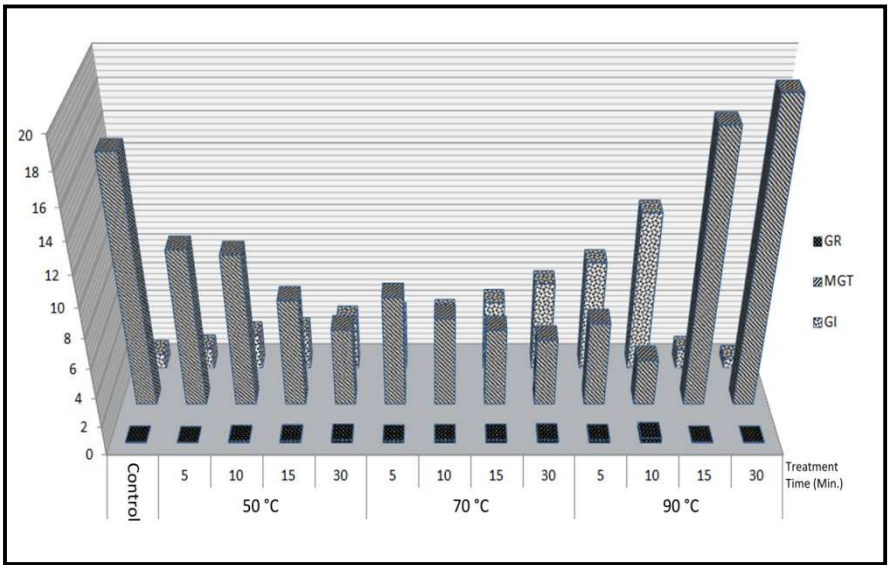

Fig. 4. The effects of different hot water treatments (50 °C, 70 °C and 90 °C) on GI, GR and MGT values of *S. kotschyana* seeds ($p < 0.05$).

GI values of the seeds treated with hot water increased in all treatment types when compared to the control. The highest increase was found in the seeds treated at 50 °C and 70 °C for 30 minutes and at 90 °C for 10 minutes. Excluding the seeds soaked in hot water at 90°C for 15 and 30 minutes, MGT values were found to decrease in all other temperature values. The highest decreases were observed in treatments at 50 and 70 °C for 30 minutes and at 90 °C for 10 minutes (Fig. 4).

The most effective treatments among the methods of dormancy breaking of *S. kotschyana* seeds were found to be mechanical scarification by hand with sandpaper and treatment with H_2SO_4. It was found that the highest germination was 100% in the seeds treated with H_2SO_4 for 180 minutes and in the seeds sandpapered by hand.

Discussion

Germination of a seed depends on development of embryo and potential of germination inhibitors. One of the most common germination inhibitor is hard and water impermeable seed coat. For this reason, the seeds of some species cannot germinate under favourable climate conditions or germination occurs in a delayed manner even if their embryos develop. Majority of *Leguminosae* species have hard and water impermeable seed coats, which inhibits seed germination and causes dormancy. Although hard seed coat is a structure which protects the embryo from mechanical effects, it has a negative impact on germination. Various scarification techniques should be applied to increase germination rates and speed of these types of seeds.

The main inhibition to water and oxygen penetration inside the *S. kotschyana* seeds is the presence of a layer of water impermeable lignified palisade cells. Similarly, this inhibition type was reported in another species [3, 6, 21, 22, 23]. Physical dormancy is observed in *S. kotschyana* seeds due to this type of seed coat. In seeds, which underwent no treatment, germination is almost totally inhibited and germination is delayed. In our study it was observed that application of various scarification techniques significantly increased germination rate. Mechanical scarification by hand with sandpaper was quite effective in increasing germination of *S. kotschyana* seeds and 100% germination was achieved. Lignified palisade cell layer in the seeds are damaged after sandpapering and germination occurs with water penetration. Similarly, it was reported that mechanical scarification by hand with sandpaper on *Medicago scutellata* and *Medicago polymorpha* [7], *Prosopis koelziana* and *Prosopis juliflora* [24] and *Capparis ovata* [25] seeds was an effective method in breaking dormancy.

Chemical scarification techniques were found to be quite effective in breaking dormancy in *S. kotschyana* seeds. In all three acid treatments (HCl, HNO_3 and H_2SO_4) germination

percentage, GI and GR values of the seeds increased when compared to the control; however MGT significantly decreased. The most effective treatment time was found to be 180 minutes in all three chemical scarification techniques. Germination rates of the seeds which underwent acid treatment varied between 10.67% and 100%. The fact that maximum germination (100%) was observed in the seeds which underwent concentrated acid treatment for a long time (180 minutes) proves that *S. kotschyana* seeds have a quite hard and thick seed coat. It was found that H_2SO_4 treatments were the most effective chemical scarification technique in breaking dormancy of the seeds of this species. Findings of the previous studies on *Prosopis koelziana* and *Prosopis juliflora* [25], *Rhynchosia capitata* [6], *Afzelia africana* [26], *Dialium guianeese* [27], *Capparis sponsa* [28], *Parika biglobosa* [11] and *Swartzia madagascariensis* [29] species about the effects of H_2SO_4 are consistent with the findings of our study. Due to high abrasiveness of H_2SO_4, hard and thick *S. kotschyana* seed coats soften; cracks occur on the coat and finally germination at a high rate occurs.

Hot water scarification techniques generally had a positive effect on germination rate. In the seeds treated with hot water at 50 °C and 70 °C, the highest germination was found in the seeds soaked in hot water for 30 minutes and in the seeds treated with hot water at 90 °C for 10 minutes. 90 °C hot water treatment on *S. kotschyana* seeds for 10 minutes might have softened hard and thick seed coat. As a result of this, germination rate might have increased due to water and oxygen penetration inside the seed. Rincon et al. (2003) reported that soaking the seeds in hot water induced seed germination; however, increasing the contact time of the seeds with hot water decreased seed germination percentage. It was observed that germination was lower than the controls in the seeds soaked in 90 °C hot water for longer than 10 minutes. 90 °C hot water treatment for 15 and 30 minutes might have created a destructive effect on the embryo and have caused the embryo to die. As a result of this, germination rate might be lower than the control group. Amusa (2011) treated *A. africana* species with 100 °C hot water for 12 and 24 hours and reported that germination rate decreased approximately by half when compared to the control group. These findings are consistent with our findings.

While mean germination time (MGT) significantly decreased in all treatments excluding 90 °C temperature for 15 and 30 minutes when compared to the control, germination rate (GR) increased. The lowest MGT value and the highest GR value was found in the seeds treated with H_2SO_4 for 180 minutes and in the seeds which were mechanically treated by hand scarification with sandpaper. It was reported that the lowest MGT and the highest GR values in *Prosopis koelziana* and *Prosopis juliflora* [24], *Medicago scutellata* and *Medicago polymorpha* [7] species were observed in the seeds treated with H_2SO_4 and sandpapering. These findings are consistent with our findings. The highest germination rate index (GI) was determined as 49.78% in the seeds treated with H_2SO_4 for 180 minutes.

Conclusions and Acknowledgement

Understanding germination requirements of endemic and rare species is one of the most important steps in the survival of these species. These species generally encounter germination problem. Findings of our study revealed that seed dormancy of *S. kotschyana*, which is an endemic species, is caused by hard and water-impermeable seed coat. A high level of germination was observed by scarification of seed coat and making it permeable to water and oxygen through various methods. It was found that chemical scarification with H_2SO_4 and mechanical scarification with sandpaper were the fastest and most effective dormancy breaking methods for *S. kotschyana* seeds. The most favourable methods in breaking dormancy of *S. kotschyana* seeds were determined for the first time in the present study. Based on the obtained data, it was concluded that these methods will be beneficial in germination of different plant types with similar dormancy.

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Table

Table 1. The effects of different dormancy breaking techniques applied to *S. kotschyana* seeds on germination percentage (GP), germination rate index (GI), mean germination time (MGT) and germination rate (GR).

Treatments	GP	GI	MGT	GR
Control	4^{a}	1.02 ± 0.04^{a}	16.67 ± 0.67^{a}	0.06 ± 0.002^{a}
Sandpaper	100^{n}	49.79 ± 0.11^{s}	2.16 ± 0.07^{l}	0.46 ± 0.014^{i}
Chemical Scarification				
HCl x 15 min	10.67 ± 1.33^{bcde}	4.31 ± 0.42^{bcdef}	7.53 ± 0.79^{c}	0.14 ± 0.016^{c}
HCl x 30 min	13.33 ± 1.02^{defg}	5.6 ± 0.41^{defgh}	5.44 ± 0.29^{efg}	0.18 ± 0.009^{def}
HCl x 60 min	13.33 ± 1.33^{defg}	5.96 ± 0.42^{efghi}	5.05 ± 0.75^{fgh}	0.21 ± 0.028^{ef}
HCl x 90 min	14.67 ± 1.01^{efgh}	6.67 ± 0.6^{ghik}	4.72 ± 0.15^{ghi}	0.21 ± 0.007^{f}
HCl x 180 min	17.33 ± 1.33^{gh}	8.13 ± 0.67^{kl}	3.63 ± 0.2^{ik}	0.28 ± 0.015^{gh}
HNO_3 x 15 min	10.67 ± 1.33^{bcde}	4.31 ± 0.57^{bcdef}	6.73 ± 0.13^{cd}	0.15 ± 0.003^{cd}
HNO_3 x 30 min	16^{fgh}	7.16 ± 0.04^{hikl}	5.17 ± 0.17^{fg}	0.19 ± 0.006^{ef}
HNO_3 x 60 min	17.33 ± 2.33^{hi}	7.78 ± 0.64^{ik}	5.02 ± 0.18^{fgh}	0.2 ± 0.007^{ef}
HNO_3 x 90 min	18.67 ± 1.33^{k}	8.36 ± 0.57^{kl}	5.13 ± 0.06^{fg}	0.2 ± 0.002^{ef}
HNO_3 x 180 min	26.67 ± 1.33^{l}	12.53 ± 0.6^{m}	3.79 ± 0.1^{hik}	0.26 ± 0.007^{gh}
H_2SO_4 x 15 min	41.33 ± 1.33^{m}	17.78 ± 0.66^{n}	6.33 ± 0.75^{cdef}	0.16 ± 0.02^{cde}
H_2SO_4 x 30 min	82.67 ± 1.33^{n}	37.6 ± 0.47^{p}	4.71 ± 0.11^{ghi}	0.21 ± 0.005^{f}
H_2SO_4 x 60 min	96 ± 2.31^{n}	45.96 ± 0.72^{r}	3.26 ± 0.33^{kl}	0.31 ± 0.02^{h}
H_2SO_4 x 90 min	100^{n}	49.6 ± 0.01^{s}	2.24^{l}	0.45 ± 0.001^{i}
H_2SO_4 x 180 min	100^{n}	49.78 ± 0.04^{s}	2.13 ± 0.03^{l}	0.47 ± 0.006^{i}
Hot water scarification				
50 °C x 5 min	4^{a}	1.42 ± 0.04^{a}	10.67 ± 0.66^{b}	0.09 ± 0.006^{b}
50 °C x 10 min	6.67 ± 1.02^{b}	2.4 ± 0.47^{b}	10.33 ± 0.33^{b}	0.1 ± 0.003^{b}
50 °C x 15 min	6.67 ± 1.33^{b}	2.71 ± 0.49^{b}	7.33 ± 0.67^{cd}	0.14 ± 0.014^{c}
50 °C x 30 min	8^{bc}	3.56 ± 0.04^{bc}	5.33 ± 0.33^{fg}	0.19 ± 0.01^{def}
70 °C x 5 min	9.33 ± 1.33^{bcd}	3.82 ± 0.62^{bcd}	7.55 ± 0.45^{c}	0.13 ± 0.008^{bc}
70 °C x 10 min	9.33 ± 1.33^{bcd}	4.04 ± 0.65^{bcde}	6.1 ± 0.49^{def}	0.17 ± 0.013^{cde}
70 °C x 15 min	10.67 ± 1.03^{bcde}	4.76 ± 0.65^{cdefg}	5.3 ± 0.4^{fg}	0.19 ± 0.015^{def}
70 °C x 30 min	13.33 ± 1.33^{defg}	6.09 ± 0.62^{fghi}	4.61 ± 0.06^{ghi}	0.22 ± 0.003^{f}
90 °C x 5 min	17.33 ± 1.33^{g}	7.56 ± 0.65^{ikl}	5.77 ± 0.23^{efg}	0.17 ± 0.007^{cdef}
90 °C x 10 min	22.67 ± 1.33^{ik}	10.89 ± 0.65^{m}	3.17 ± 0.09^{kl}	0.32 ± 0.009^{h}
90 °C x 15 min	2.67 ± 1.01^{l}	1.33 ± 0.06^{a}	18.2 ± 0.25^{m}	0.054 ± 0.02^{a}
90 °C x 30 min	1.33 ± 1.02^{m}	0.62 ± 0.07^{n}	21.3 ± 0.67^{n}	0.046 ± 0.019^{m}

The different letters are significantly different ($p < 0.05$).

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