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Improved germination conditions in *Cycas revoluta* L. by using sulfuric acid and hot water

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Abstract

Germination percentage (GP) is normally very low in *Cycas revoluta* seeds. In an effort to improve germination, omission of seed dormancy and acceleration of sexual propagation, sulfuric acid (H_2SO_4) (0, 10, 25 and 50%) and hot water (60, 80 and 100°C) pretreatments for different lengths of time were tested. An experiment was carried out using a factorial randomized complete block design (RCBD). Based on statistical analysis, significant differences were observed between various levels of sulfuric acid and hot water on germination at 5% level of probability. The highest acceleration of seed germination (171.3 days) was recorded in seeds pretreated with hot water at 80°C for 12 h. The most germination rates (GR) (95%) and germination value (GV) (44.47) were obtained in seeds pretreated with hot water at 100°C for 1 h along with 25% sulfuric acid for 2 h.

Keywords: *Cycas revolute* L., seed dormancy, sexual propagation, hard seed coat.
Abbreviations: GA_Gibberellic acid; GP_Germination Percentage; GR_Germination Rate; GV_Germination Value; MDG_Mean of Daily Germination; PV_Maximum Value.

Introduction

Cycas revoluta L. (Cycadaceae), common name; Sago Palm, is a plant native to southern Japan. Propagation of this species is either by seed or by removal of basal offsets, called "pups". *Cycas revoluta* is one of the most widely cultivated trees, grown in temperate, subtropical and tropical regions (Khalighi, 2001). Of all the cycads, *Cycas revoluta* is the most popular ornamentals. Seeds develop over the summer and are ready to be removed in January or February (Khalighi, 2001). Germination of seed of *Cycas revoluta* is hard and time consumer. Physical dormancy of seed causes delay in seed germination (Frett, 1987). Seeds start to germinate in 3-9 months and continue to germinate for periods of a year or more (Frett, 1987). Difficult germination and the slow growth of the resultant plants enhance the cost of production of cycads. Thus few growers propagate and grow cycads, resulting in a scarcity of this species (Frett, 1987). Presentation of a method for overcome to seed dormancy, increasing of percent and acceleration of germination and enhancing of emerge of radicle for seed is necessary. Some pretreatment such as mechanical and chemical scarification have been proved to promote the germination of the hard-seeded species of Cycas and some other species (Frett, 1987; Rouhi et al., 2010; Dewir et al.,). Nursery phases are an important part of the operation in the cultivation of many tropical tree crops (Ayodele, 1997). Seeds of many species belong to Cycadaceae respond to various pretreatment, including stratification, scarification, depulping and exposure to some chemical materials such as gibberellic acid (GA) (Schopmeyer, 1974; Frett, 1987; Arteca, 1995; Hartman et al., 1990; Liopa-Tsakalidi and Barouchas, 2011). Seeds of *Cycas revoluta* L. need some pretreatments such as soaking in sulfuric acid and hot water to enhance germination (Frett, 1987; Rouhi et al., 2010). These pretreatments are not always reliable with cycads (Dehghan and Yuen, 1983). Some researchers found that seeds of cycads germinated better after exposure to sulfuric acid, GA, darkness and mechanical pretreatments (Dehghan and Yuen, 1983; Frett, 1987). Seeds of some species especially those indigenous to semi-arid and tropical regions, containing hard and thick coat germinated at a higher percentage when soak in proper concentration of sulfuric acid and suitable temperature of hot water (Aliero, 2004; Salim-Azad et al., 2009; Nejadsahebi et al., 2007; Herron and Clemens, 2001; Karaboon et al., 2005; Muhammad and Amusa, 2003; Mandujano et al., 2005; Rosner and Harrington, 2003; Voiget and Tischler, 1997). With regard to importance of sexual propagation and external dormancy of *Cycas revoluta* L. seed, this investigation was carried out to introduce the best concentration of sulfuric acid and the best temperature of hot water for breaking of external seed dormancy in this important ornamental species.

Result

Significant differences were observed between various levels of sulfuric acid and hot water on germination ($p \leq 0.05$) (Tables 1, 2 and 3). The results of the treatments revealed that

Table 1. Effect of sulfuric acid on germination rate (GR), germination percentage (GP) and germination value (GV) in *Cycas revoluta* L.

Treatments	Germination rate (days)	Germination percentage (%)	Germination value
Control (0%)	313.3^{aa*}	83.3^{aa}	29.3^{aa}
H_2SO_4 (10%)	233.5^{aa}	60.0^{aa}	20.3^{a}
H_2SO_4 (25%)	233.5^{a}	61.6^{a}	25.8^{aa}
H_2SO_4 (50%)	0^{b}	0^{b}	0^{b}

* In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test.

Fig 1. Comparison of blacking seed of *Cycas revoluta* L. treated by high sulfuric acid concentration (50%, without viability) (left), and seed treated with other sulfuric acid concentrations (0, 10, and 25%, with viability) (right).

the highest GR were obtained in hot water at 80°C for 12 h, sulfuric acid at 10% for 2 h plus hot water at 80°C for 12 h, and sulfuric acid at 25% for 2 h plus hot water at 80°C for 12 h, with 171.3, 178.5 and 180 days, respectively (Tables 2 and 3). Results also indicated that the most GP were achieved in sulfuric acid at 25% for 1 h plus hot water at 100°C for 1 h, sulfuric acid at 0% for 2 h plus hot water at 100°C for 1 h, and sulfuric acid at 0% for 2 h plus hot water at 80°C for 12 h, with 95, 90 and 85%, respectively ($p \leq 0.05$) (Table 3). GR was decreased by 50% sulfuric acid. The highest GV (44.7) was obtained when seeds were soaked in sulfuric acid at 25% for 1 h plus hot water at 100°C for 1 h (Table 3). There were significant differences among hot water treatments on GP. Hot water at 100°C increased the GP for 22.5% higher than that of hot water at 80°C (Table 2). The results of mean comparison between treatments showed that cycad has seed coat dormancy. Concentrated sulfuric acid causes decreasing of GP due to penetration of sulfuric acid into the seed and its damage to embryo. Blacking of seed coat in high concentration of sulfuric acid confirms this subject (Fig. 1).

Discussion

The high GR obtained by chemical (sulfuric acid and hot water) treatments conform to those obtained for cycads and other species containing hard seed coat (Smith, 1978; Dehghan and Yuen, 1983; Peláez et al., 1992; Baes et al., 2002; Nejadsahebi et al., 2007; Rouhi et al., 2010; Dewir et al., 2011). The study of Mandujano et al. (2005) on *Opuntia rastrera*, containing hard seed coat, demonstrated that acid scarification treatments are harmful to the seeds because germination is reduced considerably compared to the control. The results of these researchers contrast to other *Opuntia* species that showed improved germination response following chemical scarification pretreatments (Pendly, 2001; Sánchez-Venegas, 1997; Olvera-Carrillo et al., 2003). Mandujano et al. (2005) and Olvera-Carrillo et al. (2003) proposed that some seeds do not show an increase in GP after soaking the seeds in chemical pretreatments, probably because these seeds do not have water-soluble inhibitors in the coat. The results obtained from our study and its comparison with other studies, revealed that the most suitable exposition time of seeds in the best concentration of chemical treatments is different among various species. Frett (1987) showed that acid sulfuric increased cycad seed germination with the greatest percent germination occurring at 1.5 h indicating that the hard seed coat poses a barrier to germination. Demel (1996) has suggested that the cause of different responses among different species to various pretreatments depends on the degree of the seed coat thickness. Many studies have shown increased cycads seed germination after scarification by acid or mechanical means (Hendricks, 1980; Smith, 1978; Dehghan and Yuen, 1983). Our results showed that hot water at 100°C increased the GP than that of hot water at 80°C. This result supports the results obtained by Muhammad and Amusa (2003) on *Tamarindus indica* L. containing hard seed coat. The results of the experiments revealed that soaking of *T. indica* seeds in hot water at 100°C for 30 min. increased GP. GP was also highest when *T. indica* seeds were soaked in 98% sulfuric acid concentration for a period of 30 min., followed by soaking in 49% sulfuric acid concentration for a period 60

Table 2. Effect of hot water on germination rate (GR), germination percentage (GP) and germination value (GV) in *Cycas revoluta* L.

Treatments	Germination rate (days)	Germination percentage (%)	Germination value
Hot water (55°C)	189.0^{aa*}	48.7^{abb}	18.3^{aa}
Hot water (80°C)	171.3^{aa}	41.2^{b}	11.5^{abb}
Hot water (100°C)	224.8^{a}	63.7^{aa}	26.7^{b}

*In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test.

Table 3. Interaction of sulfuric acid and hot water on germination rate (GR), germination percentage (GP) and germination value (GV) in *Cycas revoluta* L.

Treatments	Germination rate (days)	Germination percentage (%)	Germination value
S_0W_1	271.5^{a*}	75^{abc}	27.39^{abc}
S_0W_2	327.5^{a}	85^{ab}	28.85^{abc}
S_0W_3	341.5^{a}	90^{ab}	31.86^{ab}
S_1W_1	295.5^{a}	70^{abc}	22.57^{abc}
S_1W_2	178.5^{a}	40^{cd}	7.80^{cd}
S_1W_3	226.5^{a}	70^{abc}	30.54^{ab}
S_2W_1	189.0^{a}	50^{bc}	23.49^{abc}
S_2W_2	180.0^{a}	40^{cd}	9.55^{bcd}
S_2W_3	331.5^{a}	95^{a}	44.47^{a}
S_3W_1	0^{b}	0^{d}	0^{d}
S_3W_2	0^{b}	0^{d}	0^{d}
S_3W_3	0^{b}	0^{d}	0^{d}

* In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test.

min. GP was significantly enhanced by 50% sulfuric acid at all soaking periods. Results also indicated that seed germination increased with increasing water temperature and soaking period (Muhammad and Amusa, 2003). *Cycas* has no internal dormancy because GA treatment did not affect on cycad seed germination (Frett, 1987). It is generally thought that seed dormancy in *Cycas* is external and mechanical and chemical scarification agents are suitable for breaking seed dormancy (Hendricks, 1980; Dehghan and Yuen, 1983; Hartman et al., 1990; Arteca, 1995). Similar to *Cycas*, other species containing seed with hard coat showed high GR after mechanical and chemical (sulfuric acid) treatments (Nejadsahebi et al., 2007; Barbosa, 2005; Hartman et al., 1990; Arteca, 1995; Baes et al., 2002; Muhammad and Amusa, 2003; Mandujano et al., 2005; Olvera-Carrillo et al., 2003; Pendley, 2001; Rojas-Aréchiga and Vázquez-Yanes, 2000). The studies of Muhammad and Amusa (2003) on seed germination of *T. indica* L. with hard coat showed that the highest germination was recorded in seeds treated with 50% sulfuric acid concentration with 60 min soaking period. Germination was observed to be enhanced by increase in the sulfuric acid concentration, water temperature, and soaking period in all the trials, except with absolute sulfuric acid where poor response was observed. Many studies demonstrate that acid or mechanical scarification treatments applied to hard seeds coat permit to entrance of water and oxygen into the seeds (Sahebinejad et al., 2007; Barbosa, 2005; Herron and Clemens, 2001). According to Barbosa et al. (2005), seeds of *Strelitzia* showed better germination rate than that of control after pretreatment with 98% sulfuric acid concentration for 9 min. Scarification with sulfuric acid resulted in the better possess of water and oxygen into the seeds. Herron and Clemens (2001) showed an increased germination in order to weakling the seed coat after exposure to chemical scarification. Totally, the results of this investigation indicate that germination of *Cycas revoluta* L. seed improves after treatment with low concentration of sulfuric acid and hot water with high temperature.

Material and methods

Plant materials

The seeds of *Cycas revoluta* L. were collected from female plants growing at commercial garden located in Rasht city, northern part of Iran.

Plant treatment and cultivation

After removing the pulps mechanically, equal samples of seeds were soaked in sulfuric acid and hot water. Seeds were planted on 7 cm diameter pots in cultivation beds containing coco peat and fine sand (1:1 v/v) in 5 depths and incubated in a greenhouse at daytime temperature of 15-20°C and relative humidity of 60-70% and watered weekly as necessary. Germination was evaluated at the end of 11-12 months. Seeds with a radicle length of 6-7 cm or greater were considered to have germinated. In order to determine the effect of acid scarification pretreatments on germination depulped seeds were submerged in sulfuric acid (H_2SO_4) (0, 10, 25 and 50%, in order 2 h, 2 h, 1 h and 10 min.). Then, seeds were rinsed several times in distilled water and each batches containing 20 seeds placed in the dishes for pretreatment with hot water. The effect of hot water was studied at three temperatures, 60, 80 and 100°C, and three treatments time, 24, 12 and 1 h, respectively.

Measurements

Germination rate (GR), percent and value of germination were calculated. GR was calculated using following equation:

$$= \sum_{i=1}^{n} \frac{Si}{Di} \text{GR}$$

Where: S_i = number of germinated seed per each calculation; D_i = number of day until calculation; n = number of calculation.
Germination percentage (GP) was computed as follows:

$$GP = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \times 100$$

Germination value (GV) was obtained via following equation:
GV = PV × MDG
Where: PV = maximum value; MDG = mean of daily germination.

Data analysis

An experiment was carried out using a factorial randomized complete block design (RCBD) with 4 replications and 48 plots. Analysis of variance was done using MSTATC statistical software and means were compared using Duncan's test.

Conclusion

In conclusion, some mechanical, chemical and biological pretreatments increase acceleration of germination and breaking seed dormancy of *Cycas revoluta* L. In the case of mechanical dormancy, a restricted factor for germination, soaking of the seeds in proper concentration of sulfuric acid and proper temperature of hot water, both with the suitable lengths of time, is the best way to overcome dormancy and acceleration of germination for this species.

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