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# Effects of H<sub>2</sub>SO<sub>4</sub>, KNO<sub>3</sub> and GA<sub>3</sub> Treatments on Germination of Caper (*Capparis ovata* Desf.) Seeds

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**Abstract:** The goal of the present work is to determine the best chemical treatments to eliminate obsticles to seed germination and to stimulate growing techniques in nursery. Chemical treatments were  $H_2SO_4$  (sulfuric acid),  $GA_3$  (gibberellic acid) and  $KNO_3$  (potassium nitrate) applied for various duration and its combination. A germination percentage of 29.4% was obtained in seeds that were soaked  $H_2SO_4$  for 30 min. A germination percentage of 27.4% was obtained in seeds which were soaked 300 mg  $L^{-1}$   $GA_3$  for 3 h after treatment with  $H_2SO_4$  for 30 min and a germination percentage of 49.7% was provided by soaking seeds in 0.2%  $KNO_3$  for 8 h after treatment with  $H_2SO_4$  for 20 min.

Key words: Capparis ovata Desf, caper, germination obsticle, H<sub>2</sub>SO<sub>4</sub>, GA<sub>3</sub>, KNO<sub>3</sub>

## INTRODUCTION

There are 350 species in the genus *Capparis* L.<sup>[1,2]</sup>. *Capparis ovata* Desf., a prostrate shrub and *Capparis spinosa* L., found in most arid zones of Mediterranean countries, are called "capers"<sup>[3]</sup>. *C. ovata* and *C. spinosa* have wide natural distribution in Turkey and are found in all regions except Blacksea and Thrace<sup>[4]</sup>. In general, *C. spinosa*'s native distribution is between 200 and 300 m altitude; *C. ovata* appears naturally from 250 to 1600 m, especially in the Northeastern region of Turkey<sup>[5]</sup>.

Caper shows the characteristics of a plant adapted to poor soils, where water and nutrients are major limiting factors<sup>[6]</sup>. It has a deep root system up to 40 m<sup>[7]</sup> and short stem from which the branches grow. A C. ovata individual can achieve 1 m in height and occupy an area of 15 m<sup>2</sup>, with a canopy of 4 to 6 radial branches from which many secondary branches grow. Plants grow well in nutrient poor sharply drained gravely soils. Capers are resistant to drought and heath damage and are often seen hanging, draped and spiralling as they scramble over soil and rocks. Caper is used for soil erosion prevention in slopy areas<sup>[1,8]</sup>. The commercially valuable parts of caper are the immature flower buds, which are pickled in vinegar or preserved in granular salt. Semi-mature fruits (caperberries) and young shoots with small leaves may also be pickled for use as a condiment. Locally, capers are collected from wild plants within their natural range. Harvesting is carried out regularly throughout the growing season[9].

Although capers are widely grown on dry land where environmental conditions are difficult for the cultivation of other crops, it is difficult to propagate seedlings because of germination problems due to dormancy and hard seeds. The structure of the seed and the musilage which develops when the seed is placed in contact with water could impose an effective barrier against the diffusion of oxygen to the embryo<sup>[10]</sup>. Recently there has been some interest in growing caper as a commercial crop, but problems have arisen regarding the poor germinability of the seed<sup>[11]</sup>. Also, according to some researchers, there is germination obsticle in the caper seeds and thus there is propagation difficulties of caper seedlings<sup>[1,10]</sup>.

Germination percentage of the caper seeds is 5% and application of soaking in  $\rm H_2SO_4$  with duration of 15-30 minutes is well-known method to increase germination percentage<sup>[7]</sup>.

There are many studies and researches on germination obsticle and propagation of the seedling of *Capparis* L. by using different methods. The goal of this study was to overcome the problems of seed dormancy and to increase the germination percentage up to germination percentage of 5% at *C. ovata* by using concentrated H<sub>2</sub>SO<sub>4</sub>, KNO<sub>3</sub> and GA<sub>3</sub>.

## MATERIALS AND METHODS

Seeds of *C. ovata* were collected from natural plants located in Artvin region located in the Northeastern Turkey. The dehisced fruits were collected in September

T-1.1. 1.	Combinations	.CIT OO	- 4 (2) (0)	
Table 1:	Combinations	OT HaSUa at	na KNU	treatments

	Durations of H <sub>2</sub> SO <sub>4</sub> and KNO <sub>3</sub>		
Dose of			
KNO <sub>3</sub> (B)	10 min H <sub>2</sub> SO <sub>4</sub> (A <sub>1</sub> ) 6 h KNO <sub>3</sub> (B <sub>01</sub> )	20 min H <sub>2</sub> SO <sub>4</sub> (A <sub>2</sub> ) 6 h KNO <sub>3</sub> (B <sub>01</sub> )	30 min H <sub>2</sub> SO <sub>4</sub> (A <sub>3</sub> ) 6 h.KNO <sub>3</sub> (B <sub>01</sub> )
0.1% (B <sub>10</sub> )	$\mathrm{A_{1}B_{11}}$	$\mathrm{A_2B_{11}}$	$A_3B_{11}$
0.2% (B <sub>20</sub> )	$\mathrm{A_{1}B_{21}}$	$\mathrm{A_2B_{21}}$	$\mathrm{A_{3}B_{21}}$
0.3% (B <sub>30</sub> )	$A_1B_{31}$	$A_2B_{31}$	$A_{3}B_{31}$
	10 min H <sub>2</sub> SO <sub>4</sub> (A <sub>1</sub> ) 8 h KNO <sub>3</sub> (B <sub>02</sub> )	20 min H <sub>2</sub> SO <sub>4</sub> (A <sub>2</sub> ) 8 h KNO <sub>3</sub> (B <sub>02</sub> )	30 min H <sub>2</sub> SO <sub>4</sub> (A <sub>3</sub> ) 8 h KNO <sub>3</sub> (B <sub>02</sub> )
0.1% (B <sub>10</sub> )	$\mathrm{A_{1}B_{12}}$	$\mathrm{A_2B_{12}}$	$A_3B_{12}$
0.2% (B <sub>20</sub> )	$\mathrm{A_{1}B_{22}}$	$\mathrm{A_{2}B_{22}}$	$\mathrm{A_{3}B_{22}}$
0.3% (B <sub>30</sub> )	$\mathrm{A_{1}B_{32}}$	$\mathrm{A_{2}B_{32}}$	$A_{3}B_{32}$
	10 min H <sub>2</sub> SO <sub>4</sub> (A <sub>1</sub> ) 12 h KNO <sub>3</sub> (B <sub>03</sub> )	20 min H <sub>2</sub> SO <sub>4</sub> (A <sub>2</sub> ) 12 h KNO <sub>3</sub> (B <sub>03</sub> )	30 min H <sub>2</sub> SO <sub>4</sub> (A <sub>3</sub> ) 12 h KNO <sub>3</sub> (B <sub>03</sub>
0.1% (B <sub>10</sub> )	$A_1B_{13}$	$A_{2}B_{13}$	$A_{3}B_{13}$
0.2% (B <sub>20</sub> )	$\mathrm{A_{1}B_{23}}$	$\mathrm{A_2B_{23}}$	$A_3B_{23}$
0.3% (B <sub>30</sub> )	$A_1B_{33}$	$A_2B_{33}$	$A_{3}B_{33}$

Table 2: Combinations of H2SO4 ve GA3 treatments

Table 2: Combination	ns of H <sub>2</sub> SO <sub>4</sub> ve GA <sub>3</sub> treatments		
	Durations of H <sub>2</sub> SO <sub>4</sub> and GA <sub>3</sub>		
Dose of			
$GA_3$ (C)	10 min H <sub>2</sub> SO <sub>4</sub> (A <sub>1</sub> ) 1 h GA <sub>3</sub> (C <sub>01</sub> )	20 min H <sub>2</sub> SO <sub>4</sub> (A <sub>2</sub> ) 1 h GA <sub>3</sub> (C <sub>01</sub> )	30 min H <sub>2</sub> SO <sub>4</sub> (A <sub>3</sub> ) 1 h GA <sub>3</sub> (C <sub>01</sub> )
$100 \text{ mg L}^{-1} (C_{10})$	$A_1C_{11}$	$\mathrm{A_2C_{11}}$	$A_3C_{11}$
$200 \text{ mg L}^{-1} (C_{20})$	$\mathrm{A_{1}C_{21}}$	$\mathrm{A_2C_{21}}$	$A_3C_{21}$
300 mg L <sup>-1</sup> (C <sub>30</sub> )	$A_1C_{31}$	$ m A_2C_{31}$	$A_3C_{31}$
	10 min H <sub>2</sub> SO <sub>4</sub> (A <sub>1</sub> ) 2 h GA <sub>3</sub> (C <sub>02</sub> )	20 min H <sub>2</sub> SO <sub>4</sub> (A <sub>2</sub> ) 2 h GA <sub>3</sub> (C <sub>02</sub> )	30 min H <sub>2</sub> SO <sub>4</sub> (A <sub>3</sub> ) 2 h GA <sub>3</sub> (C <sub>02</sub> )
$100 \text{ mg L}^{-1} (C_{10})$	$\mathrm{A_{1}C_{12}}$	$ m A_2C_{12}$	$\mathrm{A_{3}C_{12}}$
$200 \text{ mg L}^{-1} (C_{20})$	$\mathrm{A_{1}C_{22}}$	$ m A_2C_{22}$	$\mathrm{A_{3}C_{22}}$
300 mg L <sup>-1</sup> (C <sub>30</sub> )	$A_1C_{32}$	$ m A_2C_{32}$	$A_3C_{32}$
	10 min H <sub>2</sub> SO <sub>4</sub> (A <sub>1</sub> ) 3 h GA <sub>3</sub> (C <sub>03</sub> )	20 min H <sub>2</sub> SO <sub>4</sub> (A <sub>2</sub> ) 3 h GA <sub>3</sub> (C <sub>03</sub> )	30 min H <sub>2</sub> SO <sub>4</sub> (A <sub>3</sub> ) 3 h GA <sub>3</sub> (C <sub>03</sub> )
$100 \text{ mg L}^{-1} (C_{10})$	$A_1C_{13}$	$ m A_2C_{13}$	$A_3C_{13}$
$200 \text{ mg L}^{-1} (C_{20})$	$A_1C_{23}$	$ m A_2C_{23}$	$A_3C_{23}$
$300 \text{ mg L}^{-1} (C_{30})$	$A_1C_{33}$	$A_2C_{33}$	$A_3C_{33}$

1999. The seeds were separated from the fruit material, rinsed in tap water, dried in shade and kept at room temperature in linen sacks.

The seeds were sown under open field conditions in polyethylene pots in the spring. The polyethylene pots were filled with growing medium composed of forest soil, creek sand and manure (1:1:1). The experimental design was a randomised complete block with 3 replications for each treatment where 40 pots were used in each replication. Pots were kept under open field conditions after sowing. Treatments were as follows:

**Application of concentrated H<sub>2</sub>SO<sub>4</sub>:** Three different durations (10, 20, 30 min) of soaking in concentrated (98%) H<sub>2</sub>SO<sub>4</sub> were applied.

**Application of concentrated H<sub>2</sub>SO<sub>4</sub>, potassium nitrate (KNO<sub>3</sub>):** The seeds were soaked at 3 different doses (0.1, 0.2 and 0.3%) and durations (6, 8, 12 h) of KNO<sub>3</sub> after applying concentrated H<sub>2</sub>SO<sub>4</sub> (10, 20, 30 min). Different abbreviations were defined for different treatments, doses and durations in order to understand the applications (Table 1). The letter A describes H<sub>2</sub>SO<sub>4</sub> treatment and the letter B describes KNO<sub>3</sub> treatment. Combinations of H<sub>2</sub>SO<sub>4</sub> and KNO<sub>3</sub> treatments are given in Table 1.

**Application of concentrated H\_2SO\_{4+} gibberillic acid** (**GA<sub>3</sub>**): The seeds were soaked at 3 different doses (100, 200, 300 mg L<sup>-1</sup>) and durations (1, 2, 3 h) of GA<sub>3</sub> after

applying concentrated  $\rm H_2SO_4$  (10, 20, 30 min). In Table 2 the letter A describes  $\rm H_2SO_4$  again and the letter C describes  $\rm GA_3$  treatments. Combinations of  $\rm H_2SO_4$  and  $\rm GA_3$  treatments are given in Table 2.

Control sowing: The experiments were terminated after 2 months due to the low rate of seed germination. Data analyses were conducted using statistical programme of SPSS 9.0. All reported values were before transformation and ANOVA and Newman Keuls tests were used to determine if the difference were significant among treatments. All differences were deemed significant at  $\alpha$ =0.05.

# RESULTS AND DISCUSSION

Caper seed could only germinate if the seed coat was destroyed, e.g. by soaking in concentrated sulphuric acid and formic acid. In previous trials, it is reported that the caper seed coat and possibly other seed parts surrounding the embryo seemed to prevent germination. Orphanos<sup>[10]</sup>, Macchia *et al.*<sup>[12]</sup> and Kara *et al.*<sup>[7]</sup> expressed

Table 3: Newman keuls test for germination percentage by H2SO4 durations Duration Germination Homogeneous Count of H<sub>2</sub>SO<sub>2</sub> percentage (%) F-ratio groups Control 120 9.8 120 17.2 14 486 10 min 20 min 120 20.4 120

<sup>\* :</sup> significant at 95% significance level

Table 4: Newman Keuls Test for germination percentage by H<sub>2</sub>SO<sub>4</sub> duration with dose and duration of KNO<sub>3</sub>

Table 4. I vewman Kedis Test for germin			ar dose and darado												
A STATE OF	Germination		ED 4			ogen	eou	s							
Application	Count	percentage	F-Ratio		oup:	S									
10 min H <sub>2</sub> SO <sub>4</sub> -%0.1 KNO <sub>3</sub> 6 h	120	4.1		*											
10 min H <sub>2</sub> SO <sub>4</sub> -%0.3 KNO <sub>3</sub> 8 h	120	5.8		*	*										
10 min H₂SO₄-%0.2 KNO₃ 12 h	120	7.6	5.123*	*	*	*									
10 min H₂SO₄-%0.2 KNO₃ 8 h	120	7.8		*	*	*									
10 min H₂SO₄-‰0.1 KNO₃ 8 h	120	8.2		*	*	*	*								
10 min H <sub>2</sub> SO <sub>4</sub> -%0.3 KNO <sub>3</sub> 12 h	120	10.2		神	*	*	*								
20 min H <sub>2</sub> SO <sub>4</sub> -%0.1 KNO <sub>3</sub> 8 h	120	10.4		計	*	*	*								
20 min H <sub>2</sub> SO <sub>4</sub> -%0.2 KNO <sub>3</sub> 12 h	120	10.6		神	*	*	*								
10 min H <sub>2</sub> SO <sub>4</sub> -%0.1 KNO <sub>3</sub> 12 h	120	11.1		*	*	*	*								
20 min H <sub>2</sub> SO <sub>4</sub> -%0.1 KNO <sub>3</sub> 12 h	120	12.1		*	*	*	*	*							
20 min H <sub>2</sub> SO <sub>4</sub> -%0.3 KNO <sub>3</sub> 12 h	120	13.8		**	*	*	*	*	*						
30 min H <sub>2</sub> SO <sub>4</sub> -%0.3 KNO <sub>3</sub> 12 h	120	17.6			*	*	*	*	*	*					
30 min H <sub>2</sub> SO <sub>4</sub> -%0.1 KNO <sub>3</sub> 12 h	120	19.4				*	90	*	*	96	*				
10 min H <sub>2</sub> SO <sub>4</sub> -%0.2 KNO <sub>3</sub> 6 h	120	21.3				**	*	*	*	*	*	*			
30 min H <sub>2</sub> SO <sub>4</sub> -%0.2 KNO <sub>3</sub> 12 h	120	21.9					*	*	*	*	*	*			
20 min H <sub>2</sub> SO <sub>4</sub> -%0.2 KNO <sub>3</sub> 6 h	120	26.0						*	*	*	*	*	*		
30 min H₂SO₄-‰0.3 KNO₃ 6 h	120	28.5							*	*	*	*	*		
30 min H₂SO₄-‰0.3 KNO₃ 8 h	120	29.8								*	*	*	*	s <b>ķ</b> c	
10 min H₂SO₄-‰0.3 KNO₃ 6 h	120	32.9								*	*	*	*	**	*
20 min H <sub>2</sub> SO <sub>4</sub> -%0.3 KNO <sub>3</sub> 6 h	120	35.8									*	*	*	*	*
30 min H₂SO₄-‰0.1 KNO₃ 6 h	120	37.4										*	*	*	*
30 min H₂SO₄-%0.2 KNO₃ 6 h	120	38.6										*	*	*	*
20 min H <sub>2</sub> SO <sub>4</sub> -%0.1 KNO <sub>3</sub> 6 h	120	40.7											*	*	*
30 min H <sub>2</sub> SO <sub>4</sub> -%0.2 KNO <sub>3</sub> 8 h	120	43.3											*	*	*
20 min H <sub>2</sub> SO <sub>4</sub> -%0.3 KNO <sub>3</sub> 8 h	120	47.3												*	*
30 min H <sub>2</sub> SO <sub>4</sub> -%0.1 KNO <sub>3</sub> 8 h	120	47.4												*	*
20 min H <sub>2</sub> SO <sub>4</sub> -%0.2 KNO <sub>3</sub> 8 h	120	49.7													*

Table 5: Newman Keuls Test for germination percentage by H<sub>2</sub>SO<sub>4</sub> duration with dose and duration of GA<sub>3</sub>

	Germination			Hon	nogene	ous
Application	Count	percentage	F-Ratio	grou	ps	
10 min H <sub>2</sub> SO <sub>4</sub> -200 mg L <sup>-1</sup> GA <sub>3</sub> 1 h	120	13.2		*		
20 min H <sub>2</sub> SO <sub>4</sub> -300 mg L <sup>-1</sup> GA <sub>3</sub> 1 h	120	14.1		*	*	
$20  \text{min H}_2 \text{SO}_4 - 300  \text{mg L}^{-1}  \text{GA}_3  2  \text{h}$	120	16.0	3.445*	*	* *	
$20  \text{min H}_2 \text{SO}_4 - 200  \text{mg L}^{-1}  \text{GA}_3  1  \text{h}$	120	16.7		*	* *	
$20  \text{min H}_2 \text{SO}_4 \text{-} 100  \text{mg L}^{-1}  \text{GA}_3  1  \text{h}$	120	17.2		*	* *	*
$30  \text{min H}_2 \text{SO}_4 \text{-} 100  \text{mg L}^{-1}  \text{GA}_3  1  \text{h}$	120	17.8		*	* *	*
10 min H <sub>2</sub> SO <sub>4</sub> -100 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	18.0		神	* *	*
10 min H <sub>2</sub> SO <sub>4</sub> -300 mg L <sup>-1</sup> GA <sub>3</sub> 2 h	120	18.8		神	* *	*
30 min H <sub>2</sub> SO <sub>4</sub> -200 mg L <sup>-1</sup> GA <sub>3</sub> 1 h	120	19.4		sic .	* *	*
10 min H <sub>2</sub> SO <sub>4</sub> -200 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	19.6		sic .	* *	*
20 min H <sub>2</sub> SO <sub>4</sub> -300 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	19.7		ole .	* *	*
$10  \text{min}  \text{H}_2 \text{SO}_4 \text{-} 300  \text{mg}  \text{L}^{-1}  \text{GA}_3  1  \text{h}$	120	20.0		**	* *	*
$20  \text{min H}_2 \text{SO}_4 \text{-} 100  \text{mg L}^{-1}  \text{GA}_3  2  \text{h}$	120	20.1		*	* *	*
20 min H <sub>2</sub> SO <sub>4</sub> -100 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	20.7		神	* *	*
30 min H <sub>2</sub> SO <sub>4</sub> -300 mg L <sup>-1</sup> GA <sub>3</sub> 1 h	120	21.1		神	* *	*
30 min H <sub>2</sub> SO <sub>4</sub> -300 mg L <sup>-1</sup> GA <sub>3</sub> 2 h	120	21.1		*	* *	*
10 min H2SO <sub>4</sub> -100 mg L <sup>-1</sup> GA <sub>3</sub> 2 h	120	21.6		*	* *	*
$10  \text{min H}_2 \text{SO}_4 - 200  \text{mg L}^{-1}  \text{GA}_3  2  \text{h}$	120	21.8		*	* *	*
$30  \text{min H}_2 \text{SO}_4 - 200  \text{mg L}^{-1}  \text{GA}_3  2  \text{h}$	120	22.2		*	* *	*
20 min H <sub>2</sub> SO <sub>4</sub> -200 mg L <sup>-1</sup> GA <sub>3</sub> 2 h	120	22.4		*	* *	*
30 min H <sub>2</sub> SO <sub>4</sub> -100 mg L <sup>-1</sup> GA <sub>3</sub> 2 h	120	23.5			* *	*
30 min H <sub>2</sub> SO <sub>4</sub> -100 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	23.6			* *	*
10 min H2SO <sub>4</sub> -100 mg L <sup>-1</sup> GA <sub>3</sub> 1 h	120	23.9			* *	*
20 min H <sub>2</sub> SO <sub>4</sub> -200 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	24.6			*	*
10 min H <sub>2</sub> SO <sub>4</sub> -300 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	25.3			*	*
30 min H <sub>2</sub> SO <sub>4</sub> -200 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	26.1			*	*
30 min H <sub>2</sub> SO <sub>4</sub> -300 mg L <sup>-1</sup> GA <sub>3</sub> 3 h	120	27.4				*
# 1 100 + 1000/ 1 100 1 1						

<sup>\* :</sup> significant at 95% significance level

that the duration of soaking in concentrated  $\mathrm{H_2SO_4}$  was effective on removing germination obsticle of the caper seeds.

The results indicated that the duration of soaking in  $H_2SO_4$  was effective on germination percentage of the seeds. It was determined that the germination percentage

was higher in seeds which were soaked in  $\mathrm{H_2SO_4}$  for different durations than the control sowing (Table 3). Generally, our findings about germination of the caper seeds confirm the results of Orphanos<sup>[10]</sup> and Barbera<sup>[1]</sup>.

In the study, germination of the caper seeds started 25 days later after sowing. The highest germination

percentage of 29.4% was determined in seeds soaked in concentrated  $\rm H_2SO_4$  for 30 min. No statistical difference in germination percentage was found between 10 min. (20.4%) and 20 min. (17.2%) soaking in  $\rm H_2SO_4$ . Only 9.8% of the control seeds germinated (Tables 3).

Maximum germination percentage of 29.4% was determined for  $H_2SO_4$  application in our study, but Orphanos<sup>[10]</sup> and Macchia *et al.*<sup>[12]</sup> determined germination percentage of 40% in seeds which were soaked in concentrated  $H_2SO_4$  for 15-30 min. (Table 3).

In all treatments, germination percentage of 49.7% was highest in seeds soaked in 0.2%  $\rm KNO_3$  for 8 h after treatment with  $\rm H_2SO_4$  for 20 min. (Table 4).  $\rm GA_3$  treatments also improved germination percentage. In  $\rm GA_3$  treatments, germination percentage of 27.4% was highest in seeds soaked in 300 mg  $\rm L^{-1}$   $\rm GA_3$  for 3 h after treatment with  $\rm H_2SO_4$  for 30 min (Table 5).

Yahyaoglu<sup>[13]</sup> proposed that seeds should be soaked in 0.2% KNO<sub>3</sub> for better germination. In this study, average of germination percentage of 23.6% in 0.2% KNO<sub>3</sub> was higher than the 0.1 and 0.3% KNO<sub>3</sub>. This value of 23.6% was higher than the value of 12.5% found by Otan and Sari<sup>[14]</sup> and 8.8% indicated by Kocabaşa <sup>[15]</sup> for *C. spinosa* L. seeds soaked in 0.2% KNO<sub>3</sub>. In addition, in the application of KNO<sub>3</sub>, duration of the 8 h increased the germination percentage of the seeds according to duration of the 6 and 12 h (Table 4).

Tonger and Tansi<sup>[16]</sup> indicated that the maximum germination percentage of 55% was obtained in the seeds of *C. ovata* scarified by P320A sandpaper thickness with GA3 solutions of 400 ppm for 2 h, but we found germination percentage of 27.4% in seeds that were soaked at 300 mg L<sup>-1</sup> GA<sub>3</sub> for 3 h after treatment with  $\rm H_2SO_4$  for 30 min.

As a growing medium in the polyethylene pots which composed forest soil, creek sand and manure (1:1:1) should be useful for propagation the seedlings like expression of Otan and Sari<sup>[14]</sup>.

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