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An Investigation on the Germination of Seeds of Kwao Kreu Kao (*Pueraria candollei* Grah. Ex Benth) as Affected by Both Water Soakings and Hot Air Oven Treatments

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Abstract: This experiment was carried out at the Faculty of Technology, Mahasarakham University, Mahasarakham 4400, Thailand to investigate effects of different water-soaking durations and hot air oven treatments on the germination of seeds of Kwao Kreu Kao (*Pueraria candollei* Grah. ex Benth) plants. The experiment was laid in a Randomised Complete Block Design with four replications. The results showed that after tested for Electrical Conductivity (EC) values for cracking of seeds, all seeds being used were at a normal condition (with an average EC value of 28.56 μS $cm^{-1}g^{-1}$) and all seeds were ready for germination. Strength on impermeability of seeds declined after soaking in water for 10 h and onwards then the treated seeds had increased in weights. However, after treated under hot air oven, dry weights of all seeds became similar. Germination percentage of all treatments was most rapid during the first three weeks of germination period and later slowly increased with time. At day 91 after sowing, T_2 gave the highest percentage of germination (52%) and the lowest was found with T_1 (control) with 31.25%. Again at day 91 after sowing, T_2 gave the highest mean value of plant numbers (16.38) and the lowest was found with T_1 (7.28). Numbers of abnormal seedlings determined at day 63 after sowing were lowest with T_2 (6.25%) and worst with T_4 (20.14%). Again at day 63 after sowing, plant height was significantly tallest with T_2 (3.88 cm) and the lowest was found with T_4 (2.71 cm). Numbers of leaves were not significantly different among the treated plants reaching a highest value of 11.25 leaves $plant^{-1}$ for T_3. It may be concluded that T_2 was the best treatment for use in germinating seeds of *Pueraria candollei* Grah. ex Benth plants. Further improvements on longevity and high percentage of germination of seeds were discussed and suggested.

Key words: Abnormal seedlings, germination, hot air oven treatments, *Pueraria candollei* Grah. ex Benth, plant numbers, plant height, water-soaking

INTRODUCTION

Kwao Kreu Kao (*Pueraria candollei* Grah. ex Benth) is one of many important medicinal herbs in Thailand. The common name of Kwao Kreu Kao is a local name in Thai and these three words indicate growth characteristics of this kind of leguminous crop, i.e., the crop is normally required its hosts to support their stems and branches when they climb up to compete for radiant energy from the sun. In the past few decades, this leguminous crop could be easily found in most upper land areas of dipterocarp forests of both Western and Northern regions of the country with an elevation ranges from 300-800 m above sea level but nowadays it becomes scarcely available due to the high demand of both inland and overseas markets (Anonymous, 2004a). Samitasiri (1998) and Nantawan and Chokchaichareon (1999) reported that its inhabitation is somewhat a kind of rainforest plants where a dense number of trees are erectly established. However, this medicinal herb plant could thrive on well in any supporting stakes where the plants could extend their stems and branches of leaves for radiant energy from the sun. Individual plants produce its single large tuber roots and each tuber root could obviously give its fresh weight up to 100 kg. Tuber roots could always anchor deeply down under the soil surface. They normally produce flowers in February-March and then formed a large number of pods, each pod has 3-5 small hard seeds and each seed has a diameter of approximately 3 mm, thus the plants scatter the seeds when dry off where nature provides their genetic survival. However, it has been advocated that in nature seeds of this leguminous plant could germinate up to 30% only due to perhaps a hard pericarp of seeds and etc.

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(Sa-Ngiam, 1979; Somporn *et al*., 2002; Suttisri, 2002; Anonymous, 2004b, 2005). Therefore, there is an urgent need to learn more about germination processes on this particular plant. This medicinal herb produces chemical substances of isoflavonoids where the substances in tuber roots could possibly recognised as a female hormone of oestrogen where it possesses an active property of phyto-oestrogen chemical. It is a group of steroid hormones and it also includes other chemical substances such as miroestrol, puerarin, mirificin, diadzin, Beta-sitosterol, coumestrol and genistein (Rintara, 2005). Apart from these chemical substances, Knight and Eden (1996) also found some other chemical substances in tuber roots of the plants such as genistein, genistin and formonoetin where these and other substances possess their properties in controlling the production of free radicles in man. The lack of oestrogen in man could possibly cause an increase in the amount of free radicles, which is harmful to human health (Anonymous, 2004b). Oestrogen also has its chemical property in delaying the deterioration of bones of those old age animals (Chutima, 2005). Nowadays, with the advancement of technology, tuber roots of the plants have been used for the extraction of medicinal substances and the products have been produced in forms of powder or solution extract where physicians endorse and prescribe them for medical purposes in extending longevity in age of man and in terms of cosmetic treatments for beauty and spa for the prevention of the rapid deterioration of physical changes in man due to aging, i.e., it helps to improve physical appearances in man to attain young looking and healthy where the substances also help to delay the loss of memory, improve blood circulation and encourage food intake with a high appetite for food intake (Rintara, 2005; Anonymous, 2006). In piggery farming, the extracted substances of this leguminous plant have been used in feed rations in order to activate growth of piglets for a rapid live weight gained with time (Anonymous, 2001; Anonymous, 2004c). Chamnanya (2005) reported that in the 2004 Thailand exported *Pueraria candollei* Grah. ex Benth in various forms overseas with an income of more than fifteen millions US Dollars and the supply of the products could not meet overseas demands. Therefore, in order to attain a large quantity of the plants and able to meet the high demand for herbal products derived from this leguminous plant, it is of tangible value to carry out more experiments on different growth aspects of the plants, particularly with the problem on the germination of seeds so that some possibilities on know how technologies in germinating seeds of this medicinal herb may be achieved.

MATERIALS AND METHODS

The experiment was conducted under glasshouse conditions at the Department of Agricultural Technology, Mahasarakham University, Northeast Thailand. The experiment aims to search for an appropriate method to be used in germinating seeds of Kwao Kreu Kao (*Pueraria candollei* Grah. Ex Benth) with the use of different water soaking hours of seeds together with the use of hot air oven treatments. Approximately 600 newly harvested seeds of *Pueraria candollei* Grah. Ex Benth plants collected from rainforests of mountainous areas of Karnjanaburi province, western region of Thailand were used. Seeds were cleaned up with the use of a blower and then only a similar size of seeds was chosen for the experiment. Before sowing, all seeds were kept in a fridge at 13±1°C with a relative humidity of approximately 40% for 8 weeks to delay the deterioration of seeds and then all seeds were tested for any leakage or some small cracks in seeds with the use of distilled water where 24 flasks were used. Each flask has a capacity of 250 mL and each flask contained an amount of 125 mL distilled water and then each of them was added with 25 seeds and then placed into a fridge at 20°C for 24 h. After taking all of the flasks out from the fridge, each of them was slightly shaken for a few seconds and then poured out the water into beakers for Electrical Conductivity (EC) determinations. An EC test was carried out with the use of EC meter Model HI 98312 of Hanna Co. Ltd., USA. The results revealed that all of the solutions gave a similar EC value where no statistical differences found with an average value of 28.56 $\mu S\ cm^{-1}\ g^{-1}$ then all of the seeds were ready for further treatments (Sutaewee, 1991). The experiment was laid in a Randomised Complete Block Design (RCBD) with 4 replications and each replication has 20 seeds and each seed was sown in each black polythene bag. The treatments used were: Control, no seed treatment was applied (T_1), soaked in water for 5 h plus hot air oven at 45°C for 30 min (T_2); soaked in water for 10 h plus hot air oven at 50°C for 30 min (T_3); soaked in water for 15 h plus hot air oven at 55°C for 30 min (T_4); soaked in water for 20 h plus hot air oven at 60°C for 30 min (T_5) and soaked in water for 25 hours plus hot air oven at 65°C for 30 min (T_6). Eighty seeds of each treatment were sown in black polythene bags and each bag has a dimension of 8×13 cm for width and height, respectively. Germinating medium consisted of sand particles and burned rice husk with a ratio of both materials of 1:1 by volume. The sand particles and burned rice husk were thoroughly mixed together and moistened up and then filled into all black polythene bags at a similar quantity for seed germination. Each seed was sown into a black polythene bag to a

depth of approximately 1 cm, i.e., each bag received only one seed. Watering was carried out twice daily, i.e., early in the morning and late in the afternoon to keep the germinating media moistened up throughout the experimental period. All of the germinating polythene bags were placed in a glasshouse where an average temperature inside the glasshouse (day and night) was approximately 31.43°C with 71.89% of relative humidity. Measurement parameters used were: (1) 20-seed weight before and after soaking in water and after hot air oven treatment, germination% from days 7 up to 91 after sowing (determined at weekly intervals but some of the data are omitted due to its similar significant effect of the treatments), (2) number of seedlings at 91 days after sowing, (3) % of abnormal seedlings and (4) plant height and number of leaves plant^{-1} at 63 days after sowing. Measurement parameters from item 2 up to item 4 were carried out at weekly intervals from days 21 up to 63. However, other collected data were not included in this paper due to their similar significant effects of the treatments as those presented in this work so they were omitted. The attained results were statistically analysed using SPSS Computer Program, version 6 (SPSS, 1999).

RESULTS

Seed weights: Mean values of 20 seeds before treated in water and hot air of all treatments ranged from 0.45 to 0.48 g for T_1 (control) and T_6, respectively (Table 1). After soaking in water, 20 seeds weighed from 0.45 to 0.60 g for T_1 and T_4, respectively. The differences were large and highly significant. When treated under hot air oven, 20 seeds weighed from 0.45 to 0.48 g for T_1 and T_6, respectively. Both untreated seeds and hot air oven treated seeds produced no significant differences found among the treatments used.

Seed germination percentages: The results on germination percentages determined at two-week intervals commencing from days 7 showed that there were no significant differences found at day 7 but at day 21 the results revealed that germination% was highest with T_2 and least with T_3 with values of 40.00 and 13.75%, respectively (Table 2). The differences were large and highly significant. At day 35, a similar trend as day 21 was achieved, i.e., T_3 gave the lowest, whilst T_2 gave the highest with values of 15.00 and 45.75%, respectively. The differences were large and highly significant. At day 49, again T_2 gave the highest and the lowest was with T_3 with values of 48.25 and 21.25%, respectively. The differences were large and highly significant. At day 63, the results showed that a similar trend as day 49 was attained, i.e., T_2 gave the highest and the lowest was with T_3 with values of 50.75 and 21.25%, respectively. The differences were large and highly significant. At day 77, some small increases in germination% in all treatments were found and the highest value was with T_2 whilst T_3 gave the lowest with values of 51.25 and 21.25%, respectively. The differences were large and highly significant. At the final tested period at day 91, the results revealed a similar trend as found with most previous sampling periods, i.e., T_2 gave the highest and the lowest was found with T_3 with values of 52.00 and 25.00, respectively.

Table 1: Mean values of weights (g) derived from 20-seed of Kwao Kreu Kao (*Pueraria candollei* Grah. Ex Benth) before and after soaking in water and after hot air oven treatments

	Weights of 20 seeds (g)		
Treatments	Before soaking in water	After soaking in water	After hot air oven treatments
T_1, Control	0.45	0.45c	0.45
T_2	0.46	0.51bc	0.46
T_3	0.45	0.52bc	0.46
T_4	0.48	0.60ab	0.51
T_5	0.47	0.58ab	0.47
T_6	0.48	0.62a	0.48
F-test	NS	**	NS
CV (%)	5.72	15.70	6.39
LSD	0.0129	0.0312	0.0149

Letter(s) within columns indicate significant differences (LSD) at probability **: p = 0.01, NS = Non Significant

Table 2: Germination percentages (%) with time (days after sowing) of seeds of Kwao Kreu Kao (*Pueraria candollei* Grah. Ex Benth) as influenced by different water soaking periods and hot air oven treatments

	Days after sowing						
Treatments	7	21	35	49	63	77	91
T_1 Control	5.00	16.50c	20.00bc	25.00bc	28.75cd	31.25b	31.25bc
T_2	8.75	40.00a	45.75a	48.25a	50.75a	51.25a	52.00a
T_3	6.25	13.75c	15.00c	21.25c	21.25d	21.25c	25.00c
T_4	6.25	27.50b	31.25b	32.50b	32.50bc	32.50b	33.75b
T_5	6.25	23.75bc	23.75bc	32.50b	35.00bc	36.25b	37.50b
T_6	7.50	22.50bc	23.75bc	33.75b	37.50b	38.75b	38.75b
F-test	NS	**	**	**	**	**	**
CV (%)	5.90	4.35	5.71	7.66	7.89	7.77	6.45
LSD	1.86	3.93	3.85	3.08	2.62	2.68	2.58

Letter(s) within columns indicate significant differences (LSD) at probability; **: p = 0.01, NS = Non Significant

Table 3: Numbers of seedlings at 91 days after sowing, abnormal seedlings (%), plant height and leaf numbers plant^{-1} of Kwao Kreu Kao (*Pueraria candollei* Grah. Ex Benth)

Treatments	No. of seedlings at day 91 after sowing	Abnormal seedlings (%)	Plant height (cm) at day 63	Leaf No. plant^{-1} at day 63
T_1, Control	7.28c	17.19	3.03b	8.50
T_2	16.38a	6.25	3.88a	10.25
T_3	5.46c	12.50	3.03b	11.25
T_4	10.01bc	20.14	2.71b	9.50
T_5	13.65ab	10.38	3.70a	8.50
T_6	10.01bc	16.07	2.90b	10.50
F-test	**	NS	*	NS
CV (%)	33.68	9.21	7.29	6.14
LSD	1.78	6.33	0.20	1.74

Letter(s) within columns indicate significant differences (LSD) at probability **: p = 0.01, *: p = 0.05, NS = Non Significant

Number of seedlings, abnormal seedling %, plant height and leaf number: With average number of seedlings at 91 days after sowing, the results showed that T_2 gave the highest and the lowest was found with T_3 with values of 16.38 and 5.46 seedlings, respectively (Table 3). The results on abnormal seedlings revealed that abnormal seedlings ranged from 6.25 to 20.14 for T_2 and T_4, respectively. There was no statistical significant found among the six treatments used. With plant height, the results showed that plant height was highest with T_2 followed by T_5 whilst the rest were similar to each other with values ranged from 3.03 to 3.88 for T_1 and T_2, respectively. The differences were large and statistical significant. For leaf number at day 63 after sowing, the results showed that leaf numbers were ranging from 8.50 to 11.25 for T_1 and T_3, respectively. There was no statistical difference found among the treated plants.

DISCUSSION

With the results on Electrical Conductivity test (EC) of seeds of Kwao Kreu Kao (*Pueraria candollei* Grah. Ex Benth) before allocated to their specific treatments, the results showed that all seeds gave an average EC value of 28.56 $\mu S\ cm^{-1}\ g^{-1}$ where the results showed no leakage of seeds due to mechanical damages was found, thus it indicated that all seeds stay alive with a normal condition and ready for germination if surrounding environmental conditions favour germination processes. Sutaewee (1991) reported that if the readings on EC testing scale indicate values within a range of 25 to 29 $\mu S\ cm^{-1}\ g^{-1}$ where it indicated that all seeds stay alive and ready for sowing. Before soaking in water, mean values of seed weights of each treatment were similar and there was no significant difference found. The results indicated that all seeds possess a similar seed size but after soaking in water under their respective treatments, seed weights were similar for the first three treatments (T_1-T_3) indicating that the seeds seemed to possess impermeable property where no quantity of water was allowed to pass through to the inner portion of seeds. This may be due to perhaps the short treated durations of the water soaking treatments (T_2 and T_3) yet it became significantly higher for the last three treatments (T_4-T_6). The results indicated that seeds of the last three treatments attained some certain amount of water when soaking duration increased. The results indicated that the strength on impermeable property of seeds was declined due to an increase in soaking duration thus seed weights were relatively increased. Nevertheless, they became similar after hot air oven treatments. This must be attributable to the loss of water in seeds due to hot air oven treatments. Hot air oven treatment of seeds was applied with an aim that heat given by the oven could possibly breakdown dormancy of seeds and altered the toughness of pericarp or seed coat of seeds to allow water to pass through when sowing. The breaking of dormancy in seeds by heat of many crops has been reported by a number of authors, e.g., Chanprasert (1995), Santipracha (1997) and Kamlert (1998) and at the same time heat treatment could have activated chemical activity in seeds by altering enzyme activity in seeds as reported by Parrotta (1992), Sopita (2003) and Olvera-Carrillo *et al.* (2003). The fluctuation of environmental temperatures has its significant effect in activating germination of seeds of many crop plants (Kamlert, 1998; Hartmann *et al.*, 2002; Kambizi *et al.*, 2006).

The results on seed germination percentages with time of Kwao Kreu Kao (*Pueraria candollei* Grah. Ex Benth) due to treatments revealed that during the first three weeks of germination period, an increase in percentages of germination was most rapid compared with the rest of the germination periods up to day 91 from sowing and the highest percentage was found with T_2 where the highest percentage persisted throughout the germination period of 91 days after sowing. The results indicated the best seed treatment method of T_2 for germination technique rather than the rest since the difference was highly significant, thus T_2 could be recognized as the best method for use in germinating seeds of *Pueraria candollei* Grah. Ex Benth. The germination percentages attained with this work were much higher than that of Supinya *et al.* (2004) where they attained germination percentages only up to 11.67. This could have been attributed to the differences in the amount of preserved food and chemical composition in seeds, which could have been better for the seeds of the present work than those of Supinya *et al.* (2004). Environmental conditions during the growth and seed filling periods of the parent plants had its significant effect on growth and seed development have been

reported by Olvera-Carrillo *et al.* (2003). They stated that seeds of *O. tomentosa* were seriously affected by El Nino conditions of the 1998 where drought conditions increased degree of hardiness of seed coats whereas the seeds collected in the 2000 gave a reverse condition due to favourable conditions for growth. A similar result has been reported with other leguminous plant species where the crop plants possess some mechanisms in avoiding damages to their life cycles when exposed to unfavourable conditions (van Staden *et al.*, 1989; Teketay, 1998). The hard seed coats normally consisted of a dense set of scleroid malpighian cells where the presence of suberin, lignin and cutin are included. These substances manifest impermeable property against the diffusion of water where they induce seed dormancy period, which could possibly be occurred for a certain period of time (Baskin and Baskin, 1998). It has been stated by Pennapa (1999) that the propagation of *Pueraria candollei* Grah. Ex Benth by seeds has not been successfully attained due to many problems. However, she stated that the use of hot water treatment could be a method to be used in reducing seed impermeability and dormancy period where the method was applicable for use as reported by Tran and Cavanagh (1984) and Patane and Gresta (2006).

With the results on average number of seedlings treatment per at 91 days after sowing, the results showed that numbers of seedlings in all treatments were relatively low with average values ranged from 7 to 16 plants for T_1 and T_2, respectively although the differences due to treatments were highly significant. This must be attributable to the poor germination of seeds due to many reasons as previously discussed. It is evidently shown that high percentages of abnormal seedlings were attained in all treatments with mean values ranged from 6.25 to 20.50% for T_2 and T_4 respectively. The high number of abnormal seedlings may be attributable to two reasons, i.e., the effect due to genetic traits and perhaps poor vigor of seeds as a result of an inadequate amount of soil nutrients, particularly at a seed filling stage of the parent plants due to high soil acidity level. High degree of soil acidity has its direct effect on the release of soil nutrients as stated by Mengel and Kirkby (1987), Miller and Donahue (1990), Suksri (1999), Pholsen (2003) and Kasikranan (2003).

For the results on plant height at day 63 after sowing, plant height was highest with T_2 followed by T_5, whilst the rest were similar. The results suggested that plant heights could possibly have been influenced by degree of seed vigor, it is noticeable that number of seedlings were higher for these two treatments (T_2 and T_5) than the rest or perhaps soil aeration in the germinating polythene bags and nutrients could have been better than the rest thus seedlings were able to grow faster than the rest. However, germinating media come from the same source so there should not be varied in terms of aeration and nutrient contents. When it comes to leaf number plant^{-1} the results indicated that there was no significant effect due to treatments found in all treated plants.

For the previous published literatures, it seems more likely that there has not been an outlet for a successful seed germination of *Pueraria candollei* Grah. Ex Benth even the use of different seed treatments of the present work. This must be attributable to many factors as previously discussed, however, it may be of interest to look into some other growth aspects of this parental legume crop, e.g., the growth of the crop in nature on mountainous areas in the tropics where soil conditions may not be most suitable for seed development. It may be possible that the crop plants have been grown under high soil acidity caused by many factors such as root respiration, rainwater, depletion of calcium and others where a number of nutrients could not be released easily from soil clay minerals or even parental materials. Thus it may be of important value to improve soil conditions around root zone of the parent plants to a pH value of 6 to 6.5 (1:2.5 soil: Water by volume) with the use of an amount of dolomite followed by the application of macronutrients and the foliage spraying of micronutrients at least four times a year, particularly during vegetative and flowering stages. This is to make sure that the legume plants received all elements needed for the production of tuber roots and seeds. The application as such may improve high tuber roots and seed yields apart from high vigorous quality of seeds, hence all seeds received most of the elements needed for a rapid germination. Suksri (1999) stated that seeds of many agronomic plants such as sunflower, castor bean, sesame, soybean and etc being produced from suitable soil conditions i.e. soil pH = 6 (1:2.5 soil: Water by volume); available nitrogen = 0.037%, available phosphorus = 53 ppm and exchangeable potassium = 80 ppm and sprayed with micronutrients twice during early growth and flowering periods significantly increased seed longevity and greater germination of seeds up to 98%. Therefore, it may be possible that if some similar soil nutrient conditions are applied to the *Pueraria candollei* Grah. Ex Benth plants then both high longevity and germination% of seeds may be obtained apart from high amount of tuber root yields. Further works should perhaps focus on nutrient uptake manipulation rather than searching for high germination % of seeds obtained from the plants from rainforest area alone. Furthermore, before any process of germination of seeds taken place, seeds should be

subjected to a specific type of machine where seed coat should be altered and tinned out in order to reduce some small outer layer of seed coat so that seeds could absorb water more rapidly when germination takes place.

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