Name of the author then initial (Sentence case: Font size 12: Bold)

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ABSTRACT

Write the following in total 500 -600 words: (Normal, Sentence case Font size: 12)

Aim:
Methodology:
Results:
Conclusion:

Key words: (Provide 5 important words about work)

INTRODUCTION

Brief introduction 1- 2 pages: (Normal, Sentence case Font size: 12)

MATERIALS AND METHODS

Brief description of pioneer methods with references or/and complete details of novel methods. 1- 2 pages: (Normal, Sentence case Font size: 12).

RESULTS AND DISCUSSIONS

Describe your results with data and supported by Tables/figures/graphs with related works by various authors. 1- 2 pages: (Normal, Sentence case Font size: 12).

CONCLUSION

Nutshell out come of this work to write one paragraph: (Normal, Sentence case Font size: 12).

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REFERRENCES

Author name, initial, year of publication, title/topic, name of the journal/book, vol.& issue/publisher name and place with edition, particular pages.

ANALYSIS OF DORMANCY BREAKING AND SEED GERMINATION FACTORS IN *CLITORIA TERNATEA* LINN.

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ABSTRACT

Aim: To observe dormancy breaking and seed germination factors in *Clitoria ternatea*. **Methodology:** In this study, the roles of some important factors involving in the germination of Clitoria ternatea seeds were analyzed by standard techniques. **Results:** Temperature is one of such factor, which seems to play a major role in controlling the germination response of *C. ternatea* seeds. Maximum *in vitro* germination was observed at 30 ˚C. Germination has been retarded at 35 ˚C. The effect of hot water treatment at 60 ˚C has also been affecting the germination in *C. ternatea*. **Conclusion:** In the present study revealed that GA3 treatment generally improved germination in *C. ternatea* (GA3 100ppm) induce germination percentage (85%). In addition, con. H_2SO_4 treatment for 5min induces 95% of seed germination, whereas 50% Sulphuric acid treatment for 5 min showed 93% of seed germination.

Key words: *C. ternatea, Gibberlic acid and Sulphuric acid.*

INTRODUCTION

Clitoria ternatea (Family: *Fabaceae)* is a very well known Ayurvedic medicine used for different ailments. *C. ternatea* is commonly called butterfly pea or shankapushpi. There are more than 50 species in *Clitoria* potentially could be an economically important genus, the most frequently reported species is *C. ternatea*. In the traditional Indian systems of medicine particularly in Ayurveda, the roots, seeds and leaves of *Clitoria ternatea* have long been widely used as a brain tonic and is believed to promote memory and intelligence, and recommended for the treatment of otalgia and eruptions (Mukherjee *et al*., 2007a). The disease preventive and health promoting approach of 'Ayurveda' takes into consideration of the whole body, mind and spirit while dealing with the maintenance of health, promotion of health and treating ailments is holistic way and finds increasing acceptability in many regions of the world (Mukherjee., 2007b). *C. ternatea* is a potential medicinal plant for enhancing learning and memory (Taranalli and Cheeramkuczhi, 2000 and Rai *et al*., 2001). Root is bitter in taste used to cure sever bronchitis, asthma. *C. ternatea* have reported number of pharmacological activities such as anxiolytic, anticonvulsant, sedative, antipyretic, anti inflammatory and analgesic (Dryaneshwar *et al*., 2010; Jain *et al*., 2003; Parimaladevi *et al*., 2003; Rai *et al*., 2001and Taranalli *et al*., 2000). It is also used in the treatment of chronic bronchitis, dropsy, goiter, leprosy, mucous disorders, sight weakness, skin diseases, sore throat and tumors. This plant species possesses unique phytochemicals with multipurpose values; mainly the plant contains several glycosides (Srivastava and Pandey, 1977).

Seed, the germ of life, has received worldwide attention due to global agricultural cooperation and the increasing need to develop good, high yielding plants (Misra *et al*., 1994). Regeneration from seeds is the most often used and cheapest method of propagation in many species (Karam *et al*, 2001). Seed germination is influenced by internal factors causing dormancy including seed coat factors, embryo factors or inhibitors (Agarwal and Dadlano, 1995). Many legumes have hard seed coat that make complete rapid and uniform germination difficult in nursery and or out in the fields (Tigabu and Oden, 2001). The various methods to overcome seed coat imposed dormancy are described in Baskin and Baskin (1998); Bewley and Black (1994); Bradbear (1988). Physical treatment's can produce coat laceration (scarification), coat microfissuration (heat, ultrasound), or coat softening and embryo stimulation (pre-chilling and soaking). Chemicals can be used to remove the waterproofing or the embryo inhibitors (NaClO and C_2H_5OH) or stimulate the growth of the embryo (GA3) (Herranzen *et al*., 1998; Miyoshi and Mil, 1998). Like many other physiological processes, seed germination is temperature dependent the optimal level of temperature for germination varies considerably between species (Demel, 1996). Therefore, to achieve optimal generation, it is necessary to have knowledge of the sub and supra optimal temperature limits. Mechanical scarification treatment like nicking the seed or

nicking the seed coat with a razor blade are also effective but generally have not been practical for large quantities of seed (Mackay *et al*., 2001).

MATERIALS AND METHODS

Seed Collection: Seeds of *C. ternatea* were collected from the Kolli Hills, Namakkal District, Tamil Nadu, India. Seeds were separated from the pods and stored in paper bags at room temperature (28±2 ˚C) under laboratory conditions (RH 30-40%) until the germination tests were performed.

Seeds were subjected to temperature, chemicals, physical and mechanical treatments. Seeds were placed on moistened cotton on sterile test tubes and incubated at thermostatically controlled incubator in the dark at 16 ˚C, 28 ˚C, 30 ˚C and 35 ˚C till the radicle emergence for the temperature treatment. The chemical treatment consisted of soaking and agitating seeds in 50ml of Sulphuric acid (10%, 20%, 30%, 40% and 50%) for 1min. In second experiment the effect of Concentrated Sulphuric acid for different time intervals (1min, 3min and 5min) was investigated. After the treatment, seeds were thoroughly rinsed with running tap water for 15min. Seeds were immersed in 250 ml flask containing 100 ml of GA_3 (100ppm, 200ppm), and kept on Orbital Shaker for overnight.

To test the effects of hot water on breaking coat imposed for dormancy, 2 treatments were performed. In this experiment, seeds were soaked in tap water for 24 h and 48 h at 28˚C; followed by, 100 ml of water was first heated up to 60˚C, 80˚C and 100˚C and taken away from the heat source. 50 seeds of each was put in seize cloth bags and then immersed in hot water. Seeds were then left in the water for over night until gradually cooled down to room temperature.

The effect of mechanical scarification was investigated by carefully removing the seed coat at the distal end. After treatments, the seeds were washed under running tap water for 15 min and followed by mercuric chloride treatment for 4 min under sterile conditions. A total of 5 replicates of *C. ternatea* seed, each were used in all treatments. The seeds were placed on moistened sterile cotton on sterile test tubes and incubated at thermostatically controlled incubator in the dark till the radicle emergence. Then they were placed on the culture racks at 20 ˚C (fluorescent lamp cool white light). They were monitored every day and moistened when dry. Seeds were considered about germinated when the radicle were about 2mm long. The germination experiment was carried out for 30 days. Final germination percentages of seed were calculated for each trial.

RESULTS AND DISCUSSION

Seed Germination Techniques

Temperature Treatments: *C. ternatea* seeds showed increase in germination as temperature increase up to 16 ºC to 30 ºC but decline thereafter. Germination was not obseerved above than 35 ºC (Fig.1). The rate of germination was faster at 30 ºC and 28 ºC followed by 16 ºC.

Chemical Treatments: Sulphuric acid treatments was effective in promoting germination in *C. ternatea* but only at the highest concentration (100%) (Table.1). Germination increased with the length of exposure, and 5min was required for achieving maximal percentage (95%) and completely removing seed coat dormancy (nil hard seed percentage). The percentage of hard seed was higher in all the other treatments (Fig.1).

Table 1: Seed germination (%) of *C. ternatea* at different treatments

Treatments	**Germination (%)**	**Hard Seed (%)**
Control	25	75
Temperature (16 ˚C)	56	44
Temperature (25 ˚C	60	40
Temperature (30 ˚C	65	35
Temperature (35 ˚C	20	80
H_2O (60 ˚C)	65	35
H_2O (80 ˚C)	60	40

H_2O (100 ˚C)	25	75
Con. H_2SO_4 (10 %)	30	70
Con. H_2SO_4 (20%)	50	50
Con. H_2SO_4 (30%)	75	30
Con. H_2SO_4 (40%)	80	20
Con. H_2SO_4 (50%)	93	07
Con. H_2SO_4 (5min) Mechanical scarification	95	05
GA3 (100 ppm)	85	15
GA3 (200 ppm)	70	30
H_2O Soaking (24h) Mechanical scarification	57	43
H_2O Soaking (48h) Mechanical scarification	45	65

Physical Treatments: Soaking in hot water poorly removed coat-imposed dormancy in *C. ternatea*. The thermal increase from 60 ºC to 100 ºC gradually, decreased germination from 40% to 25%. Consequently, the percentage of dead seed was increasing gradually (10% to 60%). None of the water soaking treatments tested at 28 ºC (24 h and 48 h) significantly enhanced germination of *C. ternatea* seeds as compared to the control. The greatest percentage of seeds apparently damaged corresponded to 48 h treatments.

Germination percentage of seeds treated with different concentrations of GA_3. The highest germination was obtained in seeds treated with 100 ppm GA_3 (85%). A trend of decreasing percentage of germination and increasing decayed percentage was observed with increasing concentration of GA_3 (Table.1).

Mechanical Scarification Treatments: Soaking in water (24 h and 48 h) followed by mechanical scarification enhanced seed germination up to 45% and 57% respectively as compared to soaking in water alone. It was clearly resulted in high percentage of hard seed (Table.1). Sulphuric acid treatment (5min) followed by mechanical scarification enhanced the seed germination (95%) to the maximum level and also decreased 5.0% in hard seed (Fig.2 and Fig.3).

The highest germination percentage (82.8%) and the best germination rate (7days) were obtained in seeds of *Colutea armena* Bias and *A. huet* which were soaked in concentrated (H_2SO_4) sulphuric acid for 30 min indicated that 40 to 60 min acid scarification was more effective in completely eliminating the hard seeds and at same time not affecting the vigour and viability of the treated seeds of *Sesbania sp* (Olmez et al., 2007). In this study Concentrated Sulphuric acid treatment for 5min induce (95%) of seed germination. And 50% Sulphuric acid treatment for 5min seed germination rate is 93%. The duration of acid treatment required to adjust depending upon the percentage of hard seeds present which varied from genotypes to genotypes. Hot water treatment was less effective and proved deleterious resulting in increased percentage of abnormal or dead seeds (Vari *et a*l., 2007). In this present study the effect of hot water treatment in *C. ternatea* poor at *60* ˚C the germination percentage (65%). Temperature is one of such factor, which seems to play a major role in controlling the germination response of *C. ternatea* seeds. Maximum germination was observed under *in vitro* conditions only at 30 ˚C. Germination was reduced at 35 ˚C. GA3 treatment generally improved germination in *C. ternatea* (GA3 100ppm) induce germination percentage (85%).

CONCLUSION

In conclusion the study revealed that dormancy in *C. ternatea* is attributed to the hard seed coat under *in vitro* conditions.

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