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An investigation into mechanism of seed dormancy in medicinal plant *Clitoria ternatea* and development of an effective method to overcome dormancy

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Abstract: Clitoria ternatea **L., a climber belonging to family Fabaceae, is mentioned in Ayurveda (ancient literature) as an important medicinal herb. Dormancy of seeds has been ascribed to hard seed coat and presence of waxy layer in it. Pre-treatment with chemical solvents thus developed as the most common method to promote germination. The study aimed to investigate the mechanism of dormancy by studying the effect of prolonged seed-dipping in water. Treatment seeds were dipped in water for different durations like 24 hours, 48 hours, 72 hours, while the seeds plated directly on moistened filter paper served as Control. The percent germination at the end of 30 days for Control, 24 hours dip, 48 hours dip, 72 hours dip was 64.00±2.30%, 70.66±1.33%, 77.33±1.33% and 93.33±2.66% respectively. Germination did not improve drastically by 24 hours and 48 hours dipping but responded significantly well to prolonged dipping of 72 hours. It indicates that either the hard seed coat is major factor controlling dormancy which gets softened by prolonged dipping or the inhibitors in seed coat, if any, are not waxy but water-soluble substances, which leach-out by water dipping. The study not only dispels the proposition of waxy layer as inhibitor but also the resultant hazardous chemical seed-dipping methods. Dipping in water also promotes rapid germination of all viable seeds within shorter duration, which would help to get plants and crop of uniform age and maturity.**

Keywords: **Seed dormancy, *Clitoria ternatea,* Germination inhibitors, Seed coat.**

I. INTRODUCTION

Clitoria ternatea L. is an ornamental, perennial climber with blue flowers, belonging to family Fabaceae, also known as Shankhapushpi and Aparajita. The species is a native of Central America [1]. *C. ternatea* is mentioned in Ayurveda (ancient Indian literature) as an important medicinal herb which helps to enhance memory and mental abilities. All plant-parts are used in preparation of medicine [2]. Other applications of the plant are as a remedy for body pains, urogenital disorders and as antidote to insect stings, snakebite [3], [4].

Seeds are the main source of propagation. Germination of seed is influenced by internal factors like hard seed coat, presence of inhibitors and external factors like light, moisture, temperature etc. [5], [6]. The hard seed coat prevents entry of water and oxygen, inhibiting germination. Seed coat impermeability to water is common feature in the Fabaceae [7]. Various methods have been employed to overcome seed coat dormancy like scarification, embryo stimulation by pre-chilling, seed-coat softening by soaking and various chemical treatments [8], [9], [10]. Presence of waxy layer in the seed coat has been claimed to be important factor for dormancy [11], [12], which perhaps lead to development of chemical treatment methods. Pre-treatment with Sulfuric acid is most commonly used method to soften seed coat, to promote germination [13]. Hence there is a need to investigate mechanism of seed dormancy for development of suitable, environment friendly methods to improve percent seed germination in *C. ternatea*.

II. MATERIAL AND METHODS

Mature, dried pods of *Clitoria ternatea* L., freshly harvested from a single climber in autumn season, were used to obtain seeds for the experiment. The harvested seeds were screened to select the healthy ones. The experiment conducted in Petri-plates, comprised one Control and 3 Treatments in triplicates. Twenty-five seed were plated in each plate. Control seeds were directly plated in Petri-plates lined with moistened filter paper on first day (Day 1) of the experiment. Treatment 1 seeds were dipped in water, in a beaker, for 24 hours on Day 1, before being plated on Day 2 on moistened filter paper. Treatment 2 seed were dipped in water for 48 hours on Day 1 and 2, before being plated similarly on Day 3. Treatment 3 seed were dipped in water for 72 hours on Day 1, 2 and 3, before being plated on Day 4. Water used for dipping seed in beaker was changed every 24 hours to avoid microbial growth and for better leaching of germination inhibitors, if any. Seed germination reading was taken in the morning, every 2 days, from Day 2 onwards, for 30 days. After Day 8, germinated seeds were removed from Petri-plates during regular observation and readings, to avoid over-crowding of seedlings (Fig. 1). Graphpad Prism 5 software was used to analyse the data for ANOVA and Tukey's test.

Fig. 1 Seed germination on Day 8 (one replicate shown)

III. RESULTS AND DISCUSSION

The percent germination after 30 days of experiment was found to be- for Control (Direct plating) 64.00±2.30%, for Treatment 1 (24 hours dip) 70.66±1.33% (not significant), for Treatment 2 (48 hours dip) 77.33±1.33% (significant), for Treatment 3 (72 hours dip) 93.33±2.66% (highly significant) at $P < 0.05$ level, according to 1way ANOVA, Tukey's post hoc test (Table I; Fig. 2). The Control seeds continued to germinate upto 28th day, while all Treatments completed germination of viable seeds early by 24th day. Germination of Control seed was slow and spread-out (Day 4 to Day 28), while germination of Treatment 3 was most rapid, within shorter span (from Day 6 to Day 20) (Table I; Fig. 3).

The Control seeds were slow in absorbing moisture from filter paper. Treatment 1 seeds, dipped in water for 24 hours, absorbed moisture quickly, were first to show seed germination on Day 3, followed by germination of Control seeds on Day 4. Treatment 2 and 3 seeds remained dipped in water and hence were devoid of air supply for 48 hours and 72 hours respectively and thus their germination was delayed for corresponding period.

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Germination is enhanced by dipping seeds in water and the increase in percent-germination is proportional to duration of dipping. Seed germination did not improve drastically by 24 hours or 48 hours dipping but responded well to dipping for longer duration of 72 hours. To produce better results, water-dipping method needs long dip of 72 hours and for proper data recording the experiment needs to continue for long period of 30 days, because many seeds germinate after first 15 days of experiment. If the experiment is terminated after 15 days, it would give inaccurate results.

Some earlier works claimed the presence of waxy layer in seed coat to be important contributor to dormancy, which lead to the development of chemical treatment methods for breaking dormancy [11], [12], [13]. Effective dormancy-breaking role of water-dipping is a strong indicator that inhibitors in seed coat, if any, are not waxy substances. Inhibitors must be water soluble and are thus leached out by water dipping. It indicates that the hard seed coat is major factor controlling dormancy of seed in *Clitoria ternatea* [5, 6]. Moreover, the use of chemicals to improve germination of plant can be hazardous for environment and human health. Hence to improve percent seed germination of this important medicinal plant, the seeds may be dipped in water for 3 days before sowing. Alternatively, one or two extra irrigations after sowing can soften the seeds and leach out inhibitors to allow improved germination. Seed-dipping also promotes germination of all viable seeds within shorter duration, which would be helpful to get plants and crop of uniform age and maturity.

TABLE I: MEAN NO. OF SEEDS GERMINATED (CUMULATIVE)

Hours dip/ Days	Control	24 h dip	48 h dip	72 h dip
Day 1	0 (Plating)	0 (Dip)	0 (Dip)	0 (Dip)
Day 2	0	0 (Plating)	0 (Dip)	0 (Dip)
Day 3	0	0.67±0.33	0 (Plating)	0 (Dip)
Day 4	1.00±0.00	1.33±0.33	2.00±0.00	0 (Plating)
Day 6	3.33±0.33	4.33±0.33	4.67±0.33	5.33±0.33
Day 8	6.00±0.00	7.67±0.67	9.00±0.58	10.00±0.58
Day 10	7.66±0.33	10.67±0.67	13.00±0.58	15.00±0.58
Day 12	9.00±0.00	12.67±0.67	15.33±0.33	18.00±0.88
Day 14	10.33±0.33	14.33±0.88	17.33±0.33	20.67±0.33
Day 16	11.67±0.33	15.00±1.15	18.00±0.00	22.00±0.58
Day 18	12.67±0.33	15.67±0.88	18.67±0.33	22.67±0.88
Day 20	13.67±0.33	16.33±0.67	19.00±0.00	23.33±0.67
Day 22	14.33±0.67	17.00±0.58	19.33±0.33	23.33±0.67
Day 24	15.00±0.58	17.67±0.33	19.33±0.33	23.33±0.67
Day 26	15.67±0.33	17.67±0.33	19.33±0.33	23.33±0.67
Day 28	16.00±0.58	17.67±0.33	19.33±0.33	23.33±0.67
Day 30	16.00±0.58	17.67±0.33	19.33±0.33**	23.33±0.67***
% germination	64.00±2.30	70.66±1.33	77.33±1.33**	93.33±2.66***

Asterik marks * indicate values significantly different as compared to Control value at P≤0. 05 level, according to 1way ANOVA, Tukey's post hoc test

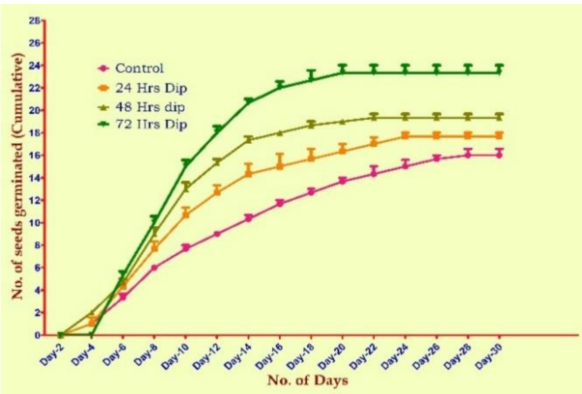

Fig. 2. Mean no. of seeds germinated (Cumulative)

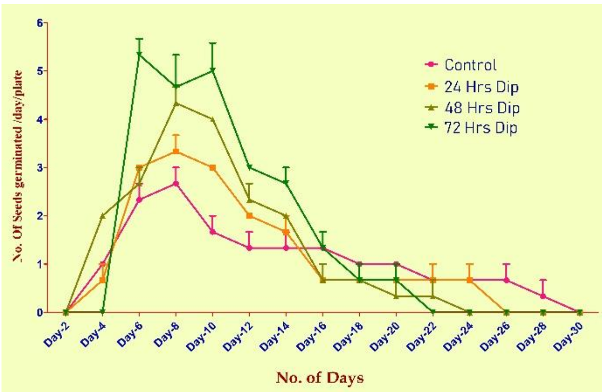

Fig. 3. Mean no. of seeds germinated per day

IV. CONCLUSION

The mechanism of seed dormancy has been confirmed to be the hardness seed coat which delays absorption of water. Germination inhibitors, if any, must be water soluble. The mechanism of presence of waxy layer in seeds proposed in earlier works and resultant development of chemical seed treatment methods are misplaced. The suitable water-dipping period for optimum germination has been standardized to be 3 days (72 hours).

Conflict of interest: The authors declare that they have no conflicts of interest regarding the research presented in this manuscript.

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