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SEED GERMINATION AND STORAGE STUDIES IN *FICUS KRISHNAE* C. DC.

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SUMMARY

***Ficus krishnae* finds its place in many aesthetic gardens due to its peculiar cup shaped leaves. Natural seed germination in the species is below 10% though the plant set seeds profusely. Seed germination studies revealed the prevalence of non-deep physiological dormancy, which could be released by scarification with conc. H_2SO_4 followed by GA_3 treatment. This has resulted in increasing seed germination to 65% with desiccated seeds sown in dark. Seeds are desiccation tolerant up to 6% moisture content and are cryostorable for long term germplasm repository.**

Key words: **Dormancy, *Ficus krishnae,* seed germination.**

INTRODUCTION

Ficus krishnae is a medium sized tree fondly known as "Krishna bor" with a legendary theme of being served as Krishna's buttercup. Though fairly distributed in native India and Pakistan, this unique fig is not well established among other tropical regions irrespective of its aesthetic leaves. Of the three leaf laminas, the central one is comparatively bigger with margins folded towards inside and found attached to the ventral mid rib to form a conical cup like structure. This kind of phyllotaxy and back- pocket differentiation is most unique in the plant kingdom. Several medicinal properties are also attributed for its juice being externally applicable for pains, rheumatism, the bark is an astringent and the seeds are considered cooling and used for making tonic (Anonymous 1956). As a common fig feature, *Ficus krishnae* also profusely set seeds. Nevertheless, the seeds are with low purity and germination percentage. Above all, as Biswas (1935) reported, *Ficus krishnae* seed germination is strange with production of only 10% true breed while the rest 90% turning out to be the horticultural variety of *F. benghalensis.* These problems together with low germination percentage (6%) and dormancy of the fresh seeds make the natural occurrence of *Ficus krishnae* meager.

There are several causes for a seed to remain dormant (Mayer and Poljakoff – Mayber 1989), which includes immaturity of embryo, impermeability of seed coat and presence of germination inhibitors. Of these a physiological process can only overcome the embryo immaturity over a period of 'after-ripening' (Bewley and Black 1985). The embryo maturation could be hastened by the use of growth regulators or subjecting seeds to effective temperatures (Bewley and Black 1985). It is reported that the pericarp tissues may contribute to dormancy by impeding gas exchange or water uptake or even restrict the loss of inhibitors via diffusion during imbibition (Bewley and Black 1994). Therefore experiments were designed to conduct studies for standardizing optimum germination pretreatments such as soaking in water, sulphuric acid, gibberellic acid and coalition treatments at various concentrations. Seeds were also desiccated to three moisture content levels and were exposed to different temperatures to determine the suitable storage conditions.

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MATERIALS AND METHODS

Multiple fruit harvesting was necessary for acquiring sufficient seeds as conspicuous fig fruit colour possessed enough attraction to disposing birds, resulting in decreased fruit number at the time of maturation similar to that reported in *Trema micrantha* (Castellani and Anguir 2001). Reddish pink fruits (synchonium) were collected from 10 year old trees of *Ficus krishnae* from the State of Kerala, India during the month of February for three consecutive years (2002, 03 and 04). Moisture content was determined as per high constant air oven method (ISTA 1985).

Germination studies:

Five replicates of 20 seeds, each placed in 9 cm diameter Petri dishes on filter paper, wetted with distilled water were kept in a seed germinator maintained at 30^0C/80% RH without light. Another set of seeds was exposed alternatively to 12 hours of 2000 Lux light and 12 hours of darkness for testing the effect of diurnal light. Seeds were also sown in sandy medium for observing the pattern and pace of germination at field conditions. In order to study the effect of coat imposed or coat containing chemical inhibitors, one seed lot was soaked in distilled water for 24 hours and another lot was chemically scarified using conc. H_2SO_4 at 2 minutes interval up to 10 minutes followed by water wash. Subjecting seeds to slow and fast desiccation by exposing to room conditions (28 ± 2^0C, 60% RH) and over silica gel in a desiccator respectively elucidated prevalence of any physiological dormancy. Seeds were also pretreated for a period of 24 hours with GA_3 100, 200, 300, 400 and 500 ppm. In another attempt to study the combinational effects, seeds were pretreated with conc. H_2SO_4 for 4 minutes followed by GA_3 300 ppm for 24 hours and *vice versa*.

Desiccation studies:

The process of slow drying was too lengthy in the sense that it took six months for 10% moisture content reduction at room conditions. Hence the seeds were dried over activated silica gel in a desiccator by considering this process as a fast desiccation method. Reduction of moisture content from the initial 24.59 to 11.9% took two-month period and was further reduced to 6.18% by continuous desiccation over silica for a period of 80 days. Seed samples at three moisture content levels were also stored for a month at different temperatures to study the effect on dormancy and viability.

Storage studies:

Fresh as well as desiccated seeds were exposed for a period of one month to different temperatures ranging from room temperature (28 ± 2^0C), 30, 20, 10, 0, -20 and -196^0C. Seed lot that was kept open to laboratory conditions (28 ± 2^0C, 60%RH), served as the control.

RESULTS

Fruits, each weighing 3.37±0.2 g, are spherical with average diameter of 1.9±0.03 cm. Polymorphism was not observed and each fruit bears 687±12.5 number of small slightly yellow seeds with an average 1000 seed weight of 0.79g. Purity analysis using seed blower per 1gm seed weight provided an average of 38.65% good seeds.

Only 6% of the fresh seeds with 23.9% initial moisture content germinated over top of paper in dark while seeds soaked for 24 hrs in distilled water germinated up to 12±4.5% germination (Table 1). Seeds

Table 1. Effect of dormancy breaking pre treatments on fresh seed germination in dark.

Pre treatments		Time	Germination (%)	Days
Control		0	6 ± 1	25.6 ± 0.4
Soaked in distilled water		24 hours	12 ± 4.5	27.2 ± 0.5
Conc. H_2SO_4		2 minutes	14 ± 1.9	27 ± 0.5
		4 minutes	22 ± 6.7	25.6 ± 0.4
		6 minutes	9 ± 4.2	27.2 ± 0.5
		8 minutes	8 ± 4.5	27.2 ± 0.5
		10 minutes	2 ± 2.7	25.6 ± 0.4
GA_3	500 ppm	24 hours	28 ± 2.7	17 ± 0.5
	400 ppm	24 hours	34 ± 5.5	20 ± 0.3
	300 ppm	24 hours	41 ± 2.2	20 ± 0.3
	200 ppm	24 hours	35 ± 3.5	21.2 ± 0.6
	100 ppm	24 hours	29 ± 2.2	22 ± 0.3

exposed to light showed 18% germination within a period of 15 days (Table 3). Germination pattern is epigeal and normally spreads for a month irrespective of laboratory or field conditions.

Scarification with conc. H_2SO_4 particularly up to 4 min interval, improved germination significantly compared to control in dark. Of all the treatments, 4 min treated seeds registered 22% germination while exceeding time limits resulted in loss of seed viability (Table 1).

Effect of pretreatments with GA_3 (from 100 to 500 ppm) showed a significant enhancement of germination (30%) in all the cases, out of which 41 % germination was obtained in dark condition in the seeds pretreated in GA_3 at 300 ppm (Table 1).

When desiccated, the germination percentage was gradually enhanced up to 20 as that of dried seeds with 6.18% moisture content. With GA_3 at 300 ppm pretreatment, even the fresh seeds registered 43% germination in dark while the germination percentage was further increased to 53 and 54% respectively with dried seeds of 11.98 and 6.18% moisture content (Table 2). All the other germination experiments related to storage temperatures and desiccation stress on seed longevity were conducted after 24 hours pretreatment with GA_3 at 300 ppm.

Table 2. Effect of desiccation and GA_3 (300 ppm) pre treatments on seed germination in dark.

Treatment	Days of desiccation	Moisture content (%)	Germination % ± SE in dark	Days taken to complete germination
Desiccated	0	23.9 ± 0.7	6 ± 1	25.6 ± 0.4
and without	60	11.98 ± 0.33	12 ± 1.2	27.2 ± 0.49
pre treatment	80	6.18 ± 0.10	20 ± 1.6	25.6 ± 0.4
LSD		0.83	3.57	1.33
Desiccated	0	23.9 ± 0.7	43 ± 2	15.2 ± 0.49
and pre treated	60	11.98 ± 0.33	53 ± 2	11.6 ± 0.4
with GA_3 300 ppm	80	6.18 ± 0.10	54 ± 1.23	17.2 ± 0.49
LSD		0.83	5.47	1.45

Compared to control value of 6%, the germination percentage in dark was doubled to 12-14 on 10% reduction of moisture content. Fresh seed germination was stimulated by light to 18% while to the seeds dried to 6.18%, light alone enhanced germination to 44%. In dark, GA_3 pretreatment enhanced the germination percentage substantially to 43 of fresh, 53 of seeds with 11.98% moisture content and 54 of seeds desiccated to 6.18% moisture content (Table 3). Nevertheless in light, germination percentage of GA_3 treated seeds was reduced in all the three tested moisture contents.

Table 3. Effect of GA_3 (300 ppm) in seeds having different moisture content in light and dark conditions.

Moisture content (%)	Treatment/Condition	Germination (%)	Days
23.9±0.7	Without GA_3 - Dark	6 ± 1	25.6 ± 0.4
	Without GA_3 - Light	18 ± 1.2	15.2 ± 0.5
	Treated with GA_3 - Dark	43 ± 2	15.2 ± 0.49
	Treated with GA_3 - Light	24 ± 1.9	21.2 ± 0.58
11.98±0.33	Without GA_3 - Dark	14 ± 5.5	25.6 ± 0.4
	Without GA_3 - Light	20 ± 1.58	25.6 ± 0.4
	Treated with GA_3 - Dark	53 ± 2	10.4 ± 0.5
	Treated with GA_3 - Light	42 ± 4.5	10.2 ± 0.4
6.18±0.10	Without GA_3 - Dark	12 ± 1.22	25.6 ± 0.4
	Without GA_3 - Light	44 ± 1.87	23 ± 0.32
	Treated with GA_3 -Dark	54 ± 2	20 ± 0.32
	Treated with GA_3 - Light	44 ± 1.87	22 ± 0.32

Combinational effects of H_2SO_4, GA_3, light and seed moisture content were tested and the effect of GA_3 was found to be antagonistic to light. Hence, only 22% germinated as against the 44% germination of acid scarified and then GA_3 pretreated fresh seeds in dark (Table 4). Maximum germination percentage of fresh seeds was 44 when pretreated with conc. H_2SO_4 followed by GA_3 in the dark. Same treatments in the case of seeds desiccated to 11.98 and 6.18 % moisture contents provided 49 and 65% germination respectively (Table 4).

Regarding the effect of desiccation and one month seed storage at different temperatures, fresh seeds with

Table 4. Combined effect of GA_3 (300 ppm) and conc. H_2SO_4 scarification in seeds having different moisture content in light and dark.

Moisture content (%)/ condition	Treatment	Germination (%)	Days
23.9 ± 0.7 Light	Scarification followed by GA_3	22 ± 2.6	20 ± 0.3
	GA_3 followed by Scarification	14 ± 1.9	25.6 ± 0.4
23.9 ± 0.7 Dark	Scarification followed by GA_3	44 ± 1.9	15.2 ± 0.95
	GA_3 followed by Scarification	28 ± 2.6	17.2 ± 0.5
11.98±0.33 Light	Scarification followed by GA_3	31 ± 1.9	17.2 ± 0.5
	GA_3 followed by Scarification	20.8 ± 0.4	20 ± 0.3
11.98±0.33 Dark	Scarification followed by GA_3	49 ± 1.9	15.2 ± 0.5
	GA_3 followed by Scarification	34 ± 1.9	17.2 ± 0.5
6.18±0.10 Light	Scarification followed by GA_3	58 ± 2.6	20 ± 0.3
	GA_3 followed by Scarification	39 ± 2.8	20 ± 0.3
6.18±0.10 Dark	Scarification followed by GA_3	65 ± 3.6	20 ± 0.3
	GA_3 followed by Scarification	48 ± 2.6	22 ± 0.3

initial moisture content of 23.9% maintained an average germination percentage of only 30%; seeds with 11.98% moisture content registered an average of 41 to 50% while seeds desiccated to 6.18% moisture content registered 50 to 52% germination (Table 5). Seeds stored for one year in polycarbonate bottles at room conditions germinated 20% with GA_3 in dark while without GA_3, only 11% germinated (Table 6). Similar results were obtained with seeds exposed to light. Seeds of the same lot that were kept open at laboratory conditions registered 65 and 43% respectively with and without GA_3 in dark. Only 40% of the light exposed seeds germinated irrespective to the pretreatments or not with GA_3 (Table 6).

Data presented as mean ± standard error on moisture content and germination percentage as well as germination period. Effect of desiccation and GA_3 at 300 ppm pretreatment on germination in dark and effect of moisture content and temperature on one-month storage were subjected to ANOVA followed with LSD test at 0.05 level.

DISCUSSION

All pretreatments during the course of this study significantly promoted germination of *Ficus krishnae* seeds compared to control. Disintegration of seed coat as well as micropylar plug in seeds treated with conc. H_2SO_4 may be the reason for imbibition and subsequent seed germination (Rolstan 1978, Egley 1989, Boscagli and Sette 2001). Seed germination of the sub genus *Urostigma* of *Ficus* was found to be favored by the soil bacteria that digest mucilaginous sticky coat (Ramirez 1976). Coat imposed dormancy was revealed by scarification of *Ficus krishnae* seeds for 4 minutes with conc. H_2SO_4 and consequent enhancement of germination in dark from 6% (control) to 22%.

Table 5. Effect of moisture content and temperature on one-month storage of *Ficus krishnae* seeds.

Sl. No.	Moisture content (%)	Germination (%) in dark, GA_3 pre treated seeds after one-month storage at different storage temperatures					
		30^0C	20^0C	10^0C	0^0C	-10^0C	-196^0C
1	23.9 ± 0.7	29 ± 4.2	31 ± 6.5	31 ± 4.2	27 ± 4.5	28 ± 4.5	28 ± 5.7
2	11.98 ± 0.33	41 ± 4.2	42 ± 4.5	43 ± 4.5	48 ± 5.7	47 ± 4.5	50 ± 3.5
3	6.18 ± 0.10	52 ± 4.5	50 ± 3.5	51 ± 4.2	50 ± 5	51 ± 4.2	53 ± 2.8
LSD	0.83	5.91	6.91	5.91	7.00	7.00	5.77

Table 6. Germination of one year stored seeds of *Ficus Krishnae.*

Condition/ Treatment	Moisture content (%)	Germination (%)	Days for germination
Poly.bottles-GA_3 300 ppm treated- Dark	9.82 ± 0.21	20 ± 1.6	20 ± 0.32
Poly.bottles-GA_3 300 ppm not treated- Dark	9.82 ± 0.21	11 ± 2.5	25.6 ± 0.4
Poly.bottles-GA_3 300 ppm treated- Light	9.82 ± 0.21	16.66 ± 1.7	22 ± 0.32
Poly.bottles-GA_3 300 ppm not treated- Light	9.82 ± 0.21	10 ± 1.7	25.6 ± 0.4
Room open-GA_3 300 ppm treated Dark	7.78 ± 0.19	65 ± 1.6	23 ± 0.32
Room open-GA_3 300 ppm not treated Dark	7.78 ± 0.19	43 ± 2.6	22 ± 0.32
Room open-GA_3 300 ppm treated Light	7.78 ± 0.19	40 ± 1.6	20 ± 0.32
Room open-GA_3 300 ppm not treated Light	7.78 ± 0.19	40 ± 1.6	23 ± 0.32

The effect of GA_3 pretreatment in breaking dormancy was reported by Robert *et al.* (1996) as dormant *Chaenorrhinum minus* seeds with 3-120 mmol L^{-1} GA_3 resulted in an enhanced 55% germination. *Sapindus trifoliatus* seed germination was enhanced from 0% (control) to 70- 89% when treated for 10 to 50 hrs at GA_3 concentrations ranging from 250 to 1500ppm (Naidu *et al.* 2000). In the present investigation, it was found that the *Ficus krishnae* seed germination in dark was promoted from 6% of the control to the 41% of pretreated seeds with GA_3 at 300ppm. It was also observed that the effect of GA_3 100ppm and the 500ppm was similar in germination of 29% barring five days earlier germination of the later case. One year stored seeds also confirm the positive role of GA_3 300 ppm application with 65% germination in dark.

Combinational pretreatments on *Ficus krishnae* seeds such as with conc. H_2SO_4 followed by GA_3 at 300 ppm provided the highest germination percentage of 65% with seeds desiccated to 6.18% moisture content sown in dark (Table 4). This result is significantly more positive when compared to the 44% germination of fresh seeds subjected to similar treatments (Table 4). These observations substantiate the cumulative role of seed dispersal by frugivores and naturally occurring GA_3 among the crevices devoid of light for favoring regeneration of *Ficus krishnae*. Research on *Zamia furfuracea* seed germination by Dehgan and Schutzman (1983) revealed the effectiveness of combined H_2SO_4 treatment for 30 min followed by 24 hour of 1000 ppm GA_3 soak. They reported that 82.2% germinated in 38 days compared to the control seeds which had only 38.8% germination in 96 days.

Significant enhancement of pretreated, scarified and desiccated *Ficus krishnae* germination in three weeks of darkness, irrespective to the differences in the nature and period of combinations showed that their embryo was fully developed. Significant enhancement in seed germination of non treated fresh seeds from 6 to 18 % and that of non treated desiccated seeds with 6.18% moisture content over 30% revealed the positive effect of light in germination (Table 3). This is akin to the feature of some non-dormant seeds that does not germinate because of absence of one or more physical environmental factors like light that are said to be in a state of enforced dormancy (Harper 1977). Light is an influencing ecological factor in regulating germination of many species (Baskin and Baskin 1998). Lisci and Pacini (1994) reported on the preference for light by the germinating non-dormant *F. carica* seeds. Seeds of many secondary species like *Ficus sur* germinate in shade though have some amount of physiological dormancy (Teketay 1993).

Ficus krishnae seeds preferred dry open storage compared to close one which in turn confirms its orthodox nature. Influence of various combinations of temperature and desiccation on storage was analysed and found that the viability was more depend on the seed moisture content rather than that of storage temperature.

This was evidenced by similar germination percentage of seeds within the three moisture content levels irrespective to the difference in storage temperatures. Seeds with high moisture content of 23.9% registered 27-31% germination after one month storage at different temperatures including –196^{0}C. Studies on *Aegle marmelos* seeds with 13.8% moisture content showed that 80% survived seven days of cryostorage (Anilkumar *et al.* 2007). *Ficus krishnae* seeds desiccated to 6.18% moisture content retained 51 to 53% germination at –10 and –196^{0}C. *Cinchona ledgeriana* seeds survived drying as low as 9% moisture content from the initial 15.5% and over 90% seeds survived one-year storage in liquid nitrogen (Som, 2000). In this experiment, one year open dry stored *Ficus krishnae* seeds with 7.7% moisture content registered 43% germination in dark without GA_3. This indicated the process of after-ripening occurred during dry storage at room temperature (Table 6). When the same seed lot of dry seeds was subjected to conc. H_2SO_4 scarification followed by GA_3 at 300ppm, 65% germination occured in dark. Based on these observations and in line with Baskin *et al*., (2006) opinion on seed dormancy, the non-deep physiological dormancy of *Ficus krishnae* was evaded. Apart from this, the cryostorage feasibility of *Ficus krishnae* seeds desiccated to 6.18% moisture content indicated the possible long term seed storage.

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