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PRE-TREATMENTS TO IMPROVE GERMINATION IN *SOLANUM VIARUM* DUNAL

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Seeds of *Solanum viarum* exhibits dormancy/hardseededness and thus complicates the recording of exact germination which is prerequisite for long term storage in the gene bank. In the present study, the initial germination percentage of seeds varied from 22% to 30% in *Solanum viarum (var. Arka sanjeevani)*. Pronounced effects of seed pretreatment with sulphuric acid and gibberelic acid on germination have been reported by the authors. The best treatments worked out was over-night soaking in GA_3 (500 ppm) and acid scarification with sulphuric acid (25%) for one hour. These treatments not only increased the germination percentage but also reduced the mean germination time significantly under laboratory conditions.

Key words : Pre-treatments, seed, *Solanum viarum*, germination, dormancy.

Steroids bearing Solanum (*Solanum viarum* Dunal syn. *S. khasianum* CB Clark var. Sengupta), is an important medicinal plant belonging to the family Solanaceae. It is a much branched prickly under-shrub or shrub distributed in the Assam Khasi hills. Mature fruits are rich source of diosgenin/solasodine, an analogue of 16-DPA, which is commercially exploited as raw material for the manufacturing of sex hormones and contraceptives. Studies on germination behaviour under laboratory conditions have not been yet done in respect to *Solanum viarum*. Seeds show poor germination (upto 30%) at optimum temperature of 25°C in dark (standerdised earlier for this genus). This may be either due to impermeable seed coat which inhibits water imbibition and thus arresting the germination or due to dormant embryo which needs triggering of hydrolytic enzymes responsible for the germination (Bewley and Black, 1978). The experiments reported here are an attempt to enhance the germination percentage through various physical and chemical treatments in laboratory conditions.

MATERIALS AND METHODS

Physiologically matured seeds of *Solanum viarum* Var. Arka sanjeevani (1994 harvest) were procured from M&AP center Bangalore through Project-Co-ordinator, Medicinal and aromatic plants. The seeds were subjected to the following dormancy breaking treatments:

a) Hot water treatment- Seeds were dipped in hot water at following combinations of temperature and time periods :

i) 60°C ... 30 min.

ii) 65°C ... 15 min.

iii) 70°C ...15 min.

iv) 75°C ... 3 min.

v) 80°C ... 1 min.

vi) 100°C ... 1 min.

After the treatments seeds were removed and cooled to room temperature.

b) Acid treatment — Seeds were subjected to the following combinations of treatments:

i) 100% Sulphuric acid ... 1 min., 5 min., 10 min.

ii) 50% Sulphuric acid ... 30 min., 60 min.

iii) 25% Sulphuric acid ... 30 min., 60 min.

After this treatment seeds were washed in running water for 1 hour.

c) Gibberelic acid (GA_3) (Pre-application) — Seeds were soaked for over-night in the following concentration of the gibberelic acid-

i) 500 ppm

ii) 100 ppm

iii) 50 ppm

d) KNO_3 — seeds were soaked for over-night in 0.2% solution.

e) GA_3 and KNO_3 were also given as co-application.

f) Acid scarified seeds (25 % H_2SO_4 for one hour) were dried to moisture level of 5.5% and stored for a fortnight. Then germination was tested.

The germination test was carried out at 25°C in dark. Twenty five seeds were placed on double layered Whatman no. 1 filter paper moistened with 5 ml of distilled water in sterilized plastic petri-plates. The percentage of normal seedlings and hard seeds were calculated from the total number of seeds placed. Mean germination time was calculated as per the formula of Ellis and Roberts (1980). Germination index is calculated as the product of radical length and germination percentage.

Germination count was taken after every 3rd day taking the protrusion of the radical as indicative of germination. Root length and shoot length of the seedlings were taken on the final day. Vigour index was calculated as the product of shoot length and germination percentage.

Data were analysed as a factorial design with minimum of four replications in all cases. All the data were subjected to an analysis of variance and were tested for significance using MSTATC Software.

RESULTS AND DISCUSSION

Statistically analysed data showed significant effects of various seed pre-treatments on germination reported in Table 1. The initial percentage of

Table 1. Effect of various pre-treatments on germination and vigour of *Solanum viarum*.

Treatment	Normal Seed-ling	Hard seed	MGT days	Total time for max. germina-tion	Root Vigour (cm.)	Shoot Vigour (cm.)	Germi-nation Index (rlx%g)	Vigour Index (shlx %g)
SULPHURIC ACID TREATMENT								
1. 100% (1 min.)	31.00	67.00	3.17	36	0.47	1.47	13.80	41.65
2. 100% (5 min)	28.00	72.00	3.15	36	0.65	1.65	18.80	46.80
3. 100% (10 min)	51.00	49.00	3.29	36	0.47	2.00	19.75	102.10
4. 50% (30 min)	28.00	71.00	6.06	36	1.15	2.00	32.40	55.60
5. 50% (60 min.)	25.00	75.00	6.12	36	1.27	1.87	34.47	47.10
6. 25% (30 min.)	72.00	28.00	13.73	21	2.12	3.67	153.80	264.20
7. 25% (60 min.)	92.00	8.00	13.47	18	3.60	4.82	330.90	443.50
GIBBERELLIC ACID PRE APPLICATION								
8. GA3 (500 PPM)	89.00	7.00	13.18	21	2.87	4.15	258.00	367.70
9. GA3 (100 PPM)	53.00	50.00	12.21	36	2.30	3.32	122.40	177.10
10. GA3 (50 PPM)	49.00	51.00	15.12	36	1.42	2.67	69.10	133.60
GA-CO-APPLICATION								
11.	58.0	42.00	17.28	36	2.60	4.15	150.80	241.50
12. Pre-treat and stored	91.00	9.00	8.058	21	2.97	4.75	272.00	430.00
13. CONTROL	31.00	67.00	13.03	36	0.62	1.77	20.67	55.50
CD at 5% level	6.73	6.88	2.48	-	0.45	0.68	44.87	60.67

dormant seeds without any treatment ranged from 65%-70% in the tested accessions. Of the twenty two treatments tried only two treatments showed positive effects i.e. above 90% germination. Immersing seeds in hot water at various combinations of temperature and time did not result in improved germination. 100% sulphuric acid treatment when given for 10 minutes, increased germination to 51% while smaller durations (1 min & 5 min.) had no effect (Fig. 1). Similar effect were seen with 50% sulphuric acid. (Table 1).

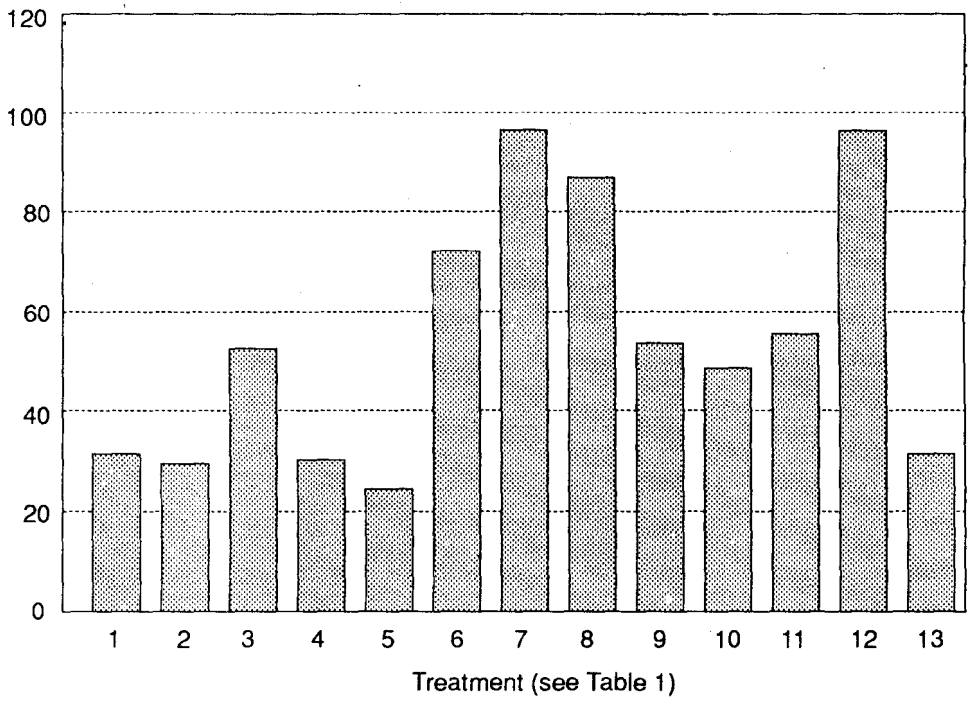

Fig. 1. Total Germination Vs. Treatments

The most successful treatment was found to be the exposure of seed to 25% sulphuric acid for one hour where germination was 100 percent. GA3 treatment for overnight in 100 ppm and 50 ppm also showed germination percentage of 50 and 53 percent respectively. Higher concentration at 500 ppm level significantly increased the germination (up to 90%). Presoaking treatment in 25% sulphuric acid for one hour where germination was 100 percent. GA3 treatment for overnight in 100 ppm and 50 ppm also showed germination percentage of 50 and 53 percent respectively. Higher concentration at 500 ppm level significantly increased the germination (up to 90%). Presoaking treatment in 25% acid for one hour and then storing seeds for a fortnight (after drying to moisture level of 5.5%), and then testing for germination was also beneficial in breaking the seed dormancy.

Rate of Germination

In the seeds without any pre-treatment (control) only 30% germination was recorded after 36 days. Various treatments resulted in reducing the total time for germination (Fig. 2). 25% Acid treatment as well as GA_3 showed

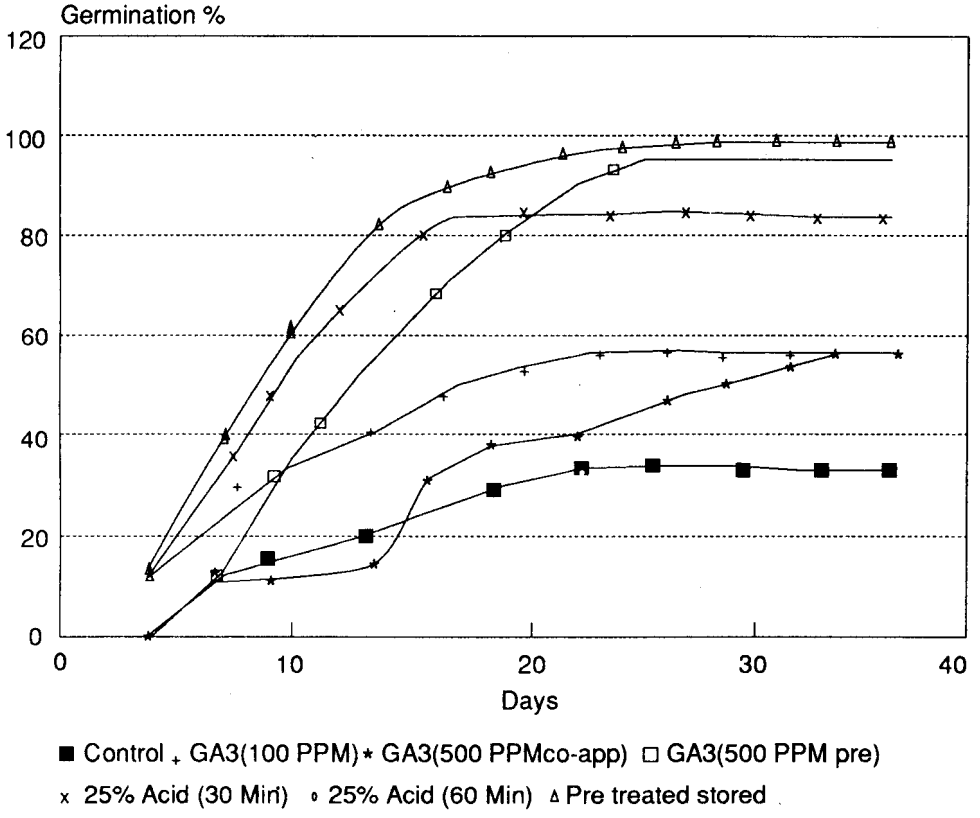

Fig. 2. Rate of Germination

100% germination and significantly reduced the mean germination time to 18-21 days. Stored pretreated seeds also took 21 days to complete the germination. Concentrated (100%) and 50% sulphuric acid treatments showed high initial germination but after 12th day it ceased. Similarly germination index was maximum in 25% acid treatment (258). The minimum germination index was recorded in 100% sulphuric acid (13.80%).

Vigour Parameter

Treatments with higher concentration of acid showed lower values for root length and shoot length and correspondingly low value of vigour index.

Maximum value of root (3.6 cm) and shoot (4.32 cm) vigour was recorded in 25% acid treatment (Table 1). Similarly vigour index was higher in 25% acid treatment (Table 1). It was followed by 25% pretreated stored seed (430.00%) and GA3 (500 ppm) with 367.7%), Interestingly all GA3 treatments were found favourable in radical and hypocotyl elongation (2.8 cm and 4.15 cm) over other treatments.

This enhancement in germination due to pre-treatments may be attributed to the easy imbition of water through scarified seed surface in acid treatment thereby facilitating the onset of germination due to mobilization of the food reserves whereas GA3 directly activates the embryo by stimulating the synthesis of proteins, m-RNA and the hydrolytic enzyme activity in the embryo (Bewlay and Black, 1978). This data is well supported by the significant improvement in the seedling vigour and rate of germination. Promotive effects of hot water treatment are reported by several authors (Padma et al 1993 in different *Acacia* spp), but in *Solanum viarum*, it had no effect in improving the germination. Sulphuric acid (100% & 50%) as well as GA3 (100 ppm & 50ppm) were less effective as compared to other successful treatments. Similar promotory effect of acid treatment and GA3 are reported by Carpenter and Boucher (1992) in different cultivars of *Catharanthus roseus*. In contrast to the findings of Rana and Nautiyal (1989) who reported that fresh acid sacrified seeds of *Acacia farnesiana* showed late onset and longer duration for completion of germination, in the present investigation acid scarification and GA3 treatment not only increased germination with an enhanced hypocotyl and radical vigour but also resulted in significant reduction in the total germination time over control.

Hence for the first time it has been worked out that seed pretreatment with 25% sulphuric acid (one hour) or with GA3 (500 ppm) resulted in 100% germination and also reduce the total germination time. This could be of great significance and of practical use in rapid multiplication of this economically important commercial medicinal plant.

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