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Substratum and temperature requirement for germination of satawar (*Asparagus racemosus*) seed

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ABSTRACT *Asparagus racemosus*, an important medicinal herb, can be propagated through seed as well as crowns (roots), but propagation through crowns is not recommended, as roots are the economic part. Seed testing protocols are also not available for medicinal plants; hence, the present investigation was carried out to work out suitable substratum and optimum temperature requirement for satawar seed germination testing. Among five substrata, 'top of the paper' and 'between the paper' were at par with each other and significantly superior to 'rolled towel papers', 'soil' and 'sand' for conducting standard germination test. Final germination percentage was non-significantly influenced by temperature, but seedling vigour parameters were significantly higher at 20°C. At 20°C temperature, germination value, speed of germination, mean germination time and days taken to initiate germination, fifty percent (T_{50}) and eighty per cent (T_{80}) germination were significantly higher than that at 25°, 30° and 35°C temperatures.

Keywords: *Asparagus racemosus*, satawar, substratum, temperature, seed germination, vigour

Asparagus racemosus Willd. an important medicinal herb belongs to family Liliaceae. Out of the 22 *Asparagus* species, *A. racemosus* is a perennial climbing shrub with fleshy roots and stems. The roots of *A. racemosus* contain several vitamins and free amino acids together with a number of antioxytosic saponins, *viz.* Shatavarin - I to IV. It is useful in the cure of nervous disorders, dyspepsia, diarrhea, tumors, inflammations, burning sensation, throat infections, tuberculosis, hypertension, abortion, agalactia, cardiac and general debility [1]. *A. racemosus* can be propagated through seed as well as crowns (roots), but propagation through crowns is not recommended, as roots are the economic part. Propagation through seeds is also advantageous over the crown in many ways. Seeds have longer shelf-life and are less bulky, easy to handle and less expensive. If sowing is delayed due to one or another reason, crowns need large and safe storage facility. Besides above, considerable time, labour and equipment are required to dig, sort and transport the crowns. In addition, there is chance to carry *Fusarium* disease from one field to another in case of propagation through seeds. Government of India enacted Seed Act for more than 95 crops so that seed sold to the farmer confirm to the minimum standards of physical purity, genetic purity, germination, seed health and moisture content for maintaining seed quality in field and for safe storage of seed.

Lack of seed testing protocols causes hindrance in tests for germination and vigour parameters of *A. racemosus* seed and ultimately results in an adverse effect on marketing. To determine standard germination of a seed lot, germination test was carried out under a given set of conditions, namely suitable substratum, optimum temperature and sufficient quantity of water to germinating seeds. Germination of *A. racemosus* has been reported to start from 15-20 days after sowing [2] to 40 days after sowing [3]. Germination percent of seeds varies from 17% to 70% [2-3]. Taking all these points into consideration, the present experiment was planned to work out suitable substratum and optimum temperature requirement for standard germination test for *A. racemosus* seeds.

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MATERIALS AND METHODS

The seeds for this study were collected from Medicinal and Aromatic Plant Research and Development Centre, Haldi, Pantnagar. The seeds were harvested in February-March 2009, cleaned manually and dried under sunlight for three days. Rough and wrinkled seeds were discarded and seeds with glossy black seed coat colour and uniform smooth surface were used in this study. Five types of substratum were evaluated to find out the best substrata for germination test of *A. racemosus*. The germination test was carried out at 20±10°C temperature in 'soil', 'sand', 'top of the paper' and 'between the paper' and 'between rolled towel paper' with four replications. In case of between the paper method, seeds were germinated in petridishes as in case of 'top of the paper' and seeds were covered with an additional moist filter paper. Prior to substratum studies, germination test was carried out at five different temperatures namely, 15±1, 20±1, 25±1, 30±1 and 35±1°C, by using 'top of the paper' method, in four replications. Final evaluation of samples were done when germination was constant for 4-5 days and seedlings were ready for evaluation. This stage was observed at 31, 38, 52 and 65 days after incubation, at 20, 25, 30 and 35°C temperatures, respectively.

Daily germination counts of seeds were made with visible radicle protrusion (~1mm) through the seed coat. At the end of test period (final count), seedlings were evaluated based on the classification of seedlings [4] and categorized in three categories (normal seedlings, abnormal seedlings and un-germinated seedlings). Seedling with a well developed root (consisting of a long and slender primary root, usually with numerous root hairs and ending in a fine tip) and a well developed shoot axis (consisting of a straight and usually slender and elongated hypocotyl) were considered as normal seedlings. Seedlings with limited damage on primary root or slight growth retardation were also included in normal seedlings (Plate1a). Seedlings with any of the essential structures (root and shoot) missing or so badly and irreparably damaged that balanced development could not be expected were categorized as abnormal (Plate1b, c and d). Seedlings with the stunned growth, week development and very thick root were also considered as abnormal seedlings. Seeds which did not germinate up to the end of test period, were categorized as un-germinated seeds. Based on daily counts and seedling evaluations, various parameters were calculated. Percent germination was calculated at the end of test period based on normal seedlings by the following formula:

$$\text{Germination}(\%) = \frac{\text{Number of normal seedlings}}{\text{Sum of normal and abnormal seedlings and ungerminated seeds}} \times 100$$

After incubation, the day on which the first germinated seed was observed, was recorded as 'days taken to germination initiation (T_I)', days taken to 50% germination (T_{50}) was calculated at the end of test period according to the formula as used by Dezfuli *et al.* [5].

$$T_{50} = ti + \frac{\{(0.8 \times N) — ni\}(ti — tj)}{(ni — nj)}$$

where, N is final number of germinated seeds. ni and nj are cumulative number of seeds germinated by adjacent counts at times ti and tj, when $ni < N/2 < nj$.

Days taken to 80% germination (T_{80}) were also calculated at the end of test period by modifying formula used for T_{50} in similar way:

$$T_{80} = ti + \frac{\{(0.8 \times N) — ni\}(ti — tj)}{(ni — nj)}$$

where, N is final number of germinated seeds. ni and nj are cumulative number of seeds germinated by adjacent counts at times ti and

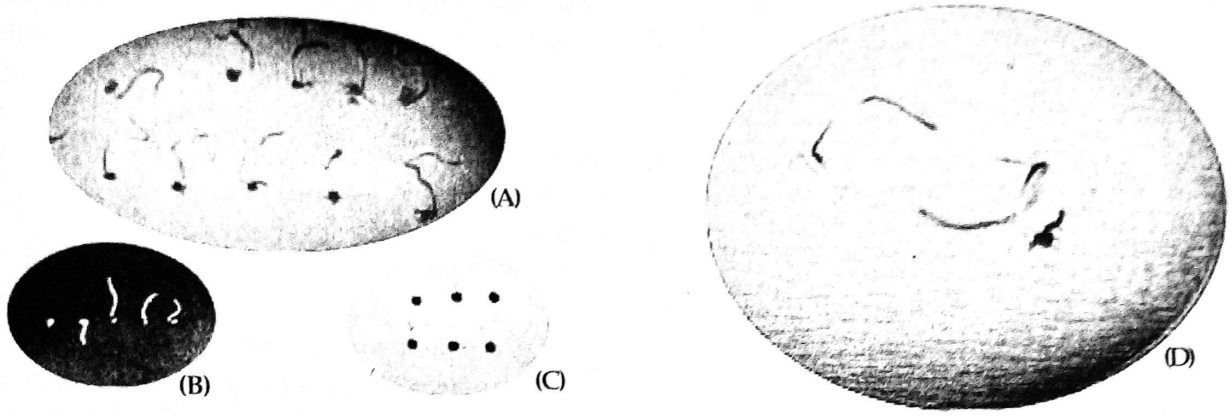

Plate 1. Normal seedlings (a) and abnormal seedlings (b, c and d) of *Satawar*

Table 1. Germination percentage of *Asparagus racemosus* as affected by different substratum

Substratum	Germination (%) †	
Soil	33.51	(30.5)
Sand	00.00	(00.0)
Top of the paper	72.17	(90.5)
Between the paper	72.35	(90.5)
Between rolled towel paper	52.58	(63.0)
SE(m)±	1.35	
CD (p=0.05)	4.07	
CV (%)	5.86	

† Values in parenthesis are original germination (%).

tj, when ni < (0.8 ×N) < nj. For calculating T_{50} and T_{80} total numbers of germinated seeds (normal + abnormal seedlings) were used as final germination. Mean germination time (MGT) was calculated by the formula [6] and speed of germination (SG) was computed [11]. The formula proposed by Djavanshir and Pourbeik [7] was used for calculating germination value (GV).

The imbibition study was also carried out at each temperature. For this, seeds were surface sterilized by dipping in 0.15% mercuric chloride solution for 10 minutes and thereafter, washing thrice with water to remove chemical adhered to seed coat. After air drying, pre-weighed 25 seeds each in four replications were weighed and placed on two moist filter papers enclosed in a petridish. These petridishes were placed in incubators at different temperatures. No fungicidal treatment was given to the seeds. The weight of 25 seeds were recorded at an interval of 2-3 days after surface drying with filter paper. Cumulative water imbibed per seed on the specific day (Di) was calculated by the following formula:

$$\text{Cumulative imbibition on Di } \left(\frac{\text{mg}}{\text{seed}}\right) = \frac{\text{Weight of seed on Di-Initial weight of seeds}}{\text{Number of seeds}}$$

The experiments were executed using completely randomized design (CRD) with four replications. The data were statistically analyzed using analysis of variance (ANOVA) appropriate for completely randomized design. In case of germination percentage, angular transformation of data was

performed before statistical analysis. Significance of null hypothesis was tested by variance ratio test at 5% level of probability. Comparison between two treatment means was made by using critical difference (CD) where 'F' test was significant [8].

RESULTS AND DISCUSSION

Effect of Substratum on germination

Seed germination of *A. racemosus* was significantly affected by substratum (Table 1). Out of five substrata tested, the highest seed germination (90.5%) was jointly recorded in between the paper and top of the paper method. Both of these were significantly superior to soil, sand and between rolled towel papers method. Based on condition prevailing during test period, it appears from data that proper aeration is very important for seed germination of *A. racemosus*. Because, in top of paper and between the paper substrata, the germination test was carried out in petridishes and these were opened on alternate days for maintaining sufficient moisture for germination. In case of 'rolled towel papers', the oxygen availability might have been restricted to the seeds due to rolling of papers as samples were not opened during entire test period. The lowest 30.5 % seed germination was recorded in soil. Seeds sown in sand did not germinate up to the evaluation of other substrata. In case of soil, the moisture supply was made by upward capillary movement of water from trays to pots in which samples were kept. This might have resulted in excess of moisture, leading to filling of soil pores with water causing reduced availability of oxygen. In case of 'sand' also, the same method of irrigation was followed and sand was kept moistened throughout study period that might have curtailed oxygen supply to seeds because of small size of sand particles (50% sand passed through 2300 mesh sieve). Although the method of irrigation was similar for both 'sand' and 'soil', but volume of soil remained the same even after watering. However, visual observations indicated that sand volume was reduced gradually with water absorption that might have reduced size of pores, which were filled with water cutting off air supply. These results are in conformity with the earlier findings in *Azadirachta indica* [9] and Palmarosa [10].

Effect of temperature on germination

Germination percentage at different temperatures ranged between 97% to 92% at 30°C and 25°C, respectively, which did not significantly differ from each other (Table 2). No germination was recorded at 15°C up to 40 days after incubation, therefore it was not considered for analysis of data. Days taken for initiation of germination at 20°C (7 days) and 25°C (7.5days) were statically at par with each other, but significantly higher than that at 30°C (17.3 days) and 35°C (19.5 days) temperatures. The number of days to initiate germination, 50% germination and mean germination time was also lowest at 20°C and gradually increased to maximum at 35°C temperature. Longer time taken for above parameters at higher temperatures can be attributed to slow rate of water imbibition at warmer temperature than at low temperature (Fig. 1). The rate of water imbibition of seed was fastest at 20°C, where it followed polynomial curve (Fig. 1a). It followed a trend of increasing at increasing rate throughout the study period. The rate of moisture imbibition at 15°C and 25°C was almost similar to that at 20°C, up to about ten days after incubation but it sharply declined. Thereafter, at 30°C and 35°C temperature, the water imbibition pattern was comparable and slower than at lower temperature (Fig. 1b). The same may be the reason for decrease in speed of germination and germination value with increase in temperature; as germination value was also significantly higher at 20°C (440) when compared to other temperature levels. The decreasing pattern of germination value

Table 2. Germination percentage and seedling vigour parameters of Asparagus racemosus seed as affected by different temperatures

Treatment	Germination (%)†	T_1 (days)	T_{50} (days)	T_{80} (days)	MGT (days)	SG	GV	
20±1°C	80.88	(96.5)	7.00	10.06	11.72	10.67	4.81	440
25±1°C	74.32	(92.0)	7.50	15.17	20.72	15.79	3.37	288
30±1°C	81.83	(97.0)	17.25	33.12	35.83	31.11	1.60	137
35±1°C	74.19	(92.5)	19.50	37.76	46.11	37.82	1.23	100
SE(m)±	2.85		1.42	0.69	0.57	0.74	0.10	13
CD (p=0.05)	NS		4.37	2.12	1.75	2.29	0.31	41
CV (%)	7.32		22.16	5.72	3.97	6.25	7.29	11

† Values in parenthesis are original germination (%); T_1, T_{50} & T_{80} are days to initiation, 50% and 80% germination; MGT = mean germination time; SG = speed of germination and GV = germination value.

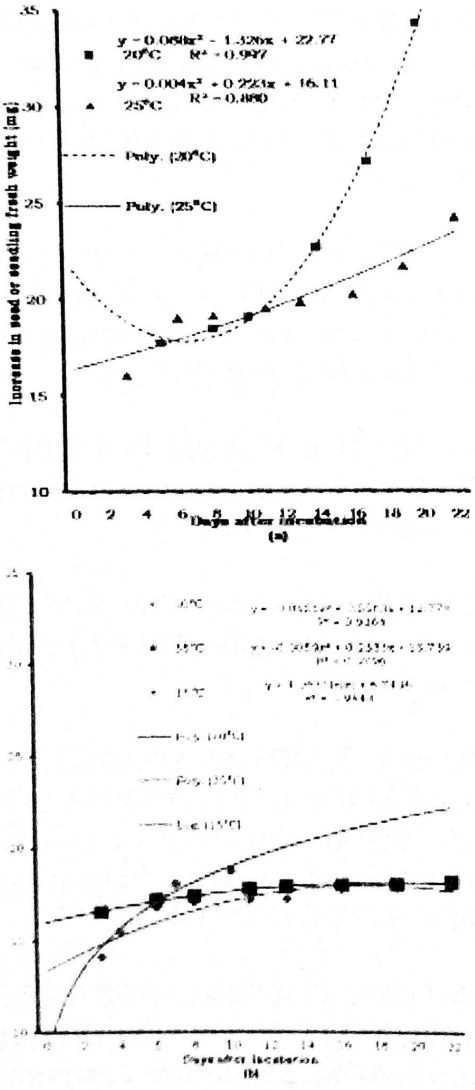

Fig. 1. Increase in fresh weight of seed (or seedling) of *Asparagus racemosus* at 20°C and 25°C (a), and 15°C, 30°C and 35°C.

with increasing temperature can be clearly seen in Fig 2. Imbibition of water initiates gibberellins biosynthesis, which is responsible for production of amylase enzymes. These enzymes are responsible for breakdown of reserve food material, especially carbohydrates. The energy and carbon skeleton needed for different anabolic processes of germination is made available by respiratory breakdown of storage compounds. Although, the enzymatic breakdown of reserve food material is temperature sensitive, but water is the basic requirement to initiate the process which being a limiting factor might have slowed down germination process at higher temperatures as compared to 20°C. The negligible information regarding optimum temperature for *A. racemosus* germination in literature suggests that the seed can germinate in three to six weeks at 25°C [14]. In present study also, 80% seed sprouting was recorded in a period of three weeks at 25°C, but at 20°C, 80% sprouting was achieved in less than two weeks, which is lower than that at 25°C. In *A. officinalis*, 30°C was better than 10°C temperature for seed germination as published in earlier report [11]. The difference between *A. racemosus* in present study and *A. officinalis* for temperature requirement may be due to difference in their genetic make-up.

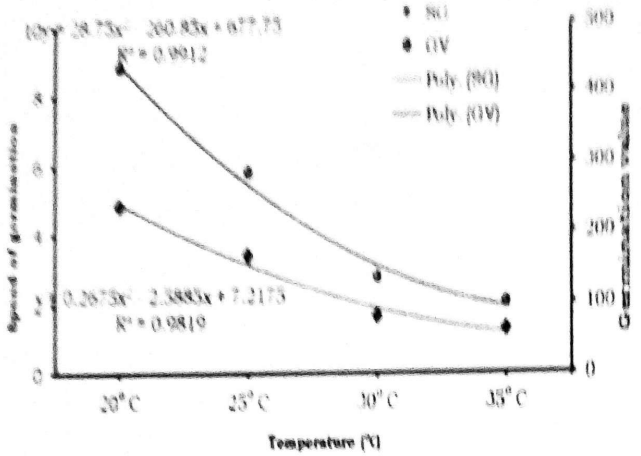

Fig. 2. Speed of germination of *Asparagus racemosus* seed at different temperatures.

Usually, the rate of water imbibition by seed increases with temperature because water imbibition being a physical process increases with temperature. Nevertheless, in the present study the pattern of water imbibition in relation to temperature was exactly reverse. It indicates that the permeability of water through seed coat is adversely influenced at warm temperature, which is possible by the presence of substance(s)* in the seed coat and/or micropyle which expand at temperature above 25°C leading to the reduction in the size of the pores or inter cellular spaces in the cell wall through which water may pass and reach endosperm. This may reduce water movement through seed coat and/or micropyle. At 15°C, the rate of water absorption recorded for ten days was comparable to that of 20°C. The present study revealed that germination test of *A. racemosus* seed should be carried out at 20°C by using top of the paper or between the paper method.

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