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Asian Journal of Plant Science and Research, 2012, 2 (4):409-413

ISSN : 2249-7412
CODEN (USA): AJPSKY

Seed germination behaviour of *Asparagus racemosus* (Shatavari) under *in-vivo* and *in-vitro* conditions

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ABSTRACT

Asparagus racemosus is an important medicinal plant, which is used in many Ayurvedic, Homeopathic and Unani preparations. The present article deals with an evaluation of its seed germination behaviour and seedling growth performance under in-vitro and in-vivo conditions. Data on various parameters such as seed morphology, germination performance influenced by different pretreatments, vigour index, seedling quality index (SQI), sowing depths and soil mixture ratios were observed. Results revealed that seeds exhibited cent percent germination under controlled laboratory conditions. Maximum SQI values were obtained when seeds were sown at 0.5 cm depth and 1:2:1 soil mixture ratios of sand: clay: FYM.

Key Words: *Asparagus racemosus*, seed germination, vigour index, seedling growth, Indian arid zone

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INTRODUCTION

The Indian Thar desert is unique both from floristic and climatic points of view. In deserts, water demand by vegetation and crops is high but availability is restricted by scanty rainfall and long dry periods throughout the year. There is a strong belief that the degradation of soil and vegetation in the arid lands has caused decreasing precipitation and droughts [1]. Seed germination in plants is a critical period determining further perpetuation of the species in natural habitat or their elimination from the area. Its response has a direct impact on a species distribution and abundance, since it is a key element affecting population dynamics especially in semi-arid environments [2].

Roots of *Asparagus racemosus* (Shatavari; Family: Asparagaceae) is used as a remedy for tuberculosis, leprosy, epilepsy, dysentery, tumors, inflammations and night blindness. Fresh juice from tuber is given orally in dysentery, acidity and to increase the breast milk after delivery. Medicated oil prepared from tubers is beneficial for nervous and rheumatic complaints [3].

The present investigation has therefore been aimed to evaluate germplasm collected from plants growing in wild and cultivated habitats to find out the effect of different growth regulators (GA_3, IAA and IBA) on various parameters of seed germination. The emphasis was also given on morphological parameters of seeds and different seeds sowing techniques such as depth, soil mixture ratios, etc. to improve germination and seedling growth under controlled laboratory (*in-vitro*) and nursery (*in-vivo)* conditions.

MATERIALS AND METHODS

(i) Morphological variations and analyses of soil and plant parameters: To record morphological variations in plants, monthly phenological observations at two selected sites, *viz.* site- I (Botanic Garden, JNV University,

Jodhpur) and site-II (Kailana Machia Reserve Park; 11 km away from University Campus in west direction) were undertaken for two consecutive years (2008-09 & 2009-10). For estimation of oil, the air-dried seeds were extracted with petroleum ether in Soxhlet apparatus for 8 h. The oil content in seeds was calculated in percentage on dry weight basis. The total (soluble & insoluble) sugars in fruit pulp were estimated by the Anthrone reagent [4]. The electrical conductivity (EC) and pH of soil collected underneath plants at both sites were measured as per standard methods.

(ii) **Seed morphology and germination behaviour under *in-vitro* conditions:** Mature fruits were collected from site-I during December to February, while that of from site-II during January to March 2008-2010. The mature fruits were cleaned and seeds were stored in plastic containers with insecticides/parad tablet. The seed viability was tested by the tetrazolium method [5]. The seed size was measured with the help of vernier caliper and graph paper. The seed volume and density were measured as per standard methods and calculated for 100 seeds in triplicate and confirmed twice. The seeds were presoaked for 24 h in different concentrations (0-10 mgl^{-1}) of growth regulators such as Gibberellic acid (GA_3), Indole Butyric Acid (IBA) and Indole Acetic Acid (IAA). After that, these were kept for germination studies. The germination experiments were performed in alternate white light and dark (12h) obtained from three fluorescent tubes of 40 watts each fitted at a height of half meter from the petridishes (1000 Lux) at 28 ^{o}C in seed germinator. For germination studies, seeds were placed in sterilized petridishes lined with single layer of filter paper. The filter paper was moistened with the required volume of distilled water as and when needed. After one week of setting the experiments, seeds germination percentage and root & shoot lengths of seedling were recorded. The seedling vigour index (VI) and germination value (GV) was calculated as proposed by Abdul-Baki and Anderson [6]. GV of the seeds, which is an index combining the speed and completeness of the germination was calculated for each treatment using the formula of Czabator [7]. The experiments were performed in triplicate with each petridish containing 10 seeds and repeated three times for confirmation of results.

(iii) **Seed germination behaviour under *in-vivo* conditions:** The nursery experiments with different soil mixture ratios and sowing depths were carried out during July 2009 & 2010. To assess the effect of different growth regulators on germination behavior and root & shoot lengths, the seeds were presoaked in GA_3 (5 mg l^{-1}) solutions. The pretreated seeds were sown in polybags with four different types of soil mixture ratios of sand: clay: FYM, *i.e.* R_1 (1:1:1), R_2 (1:2:1), R_3 (2:2:1) and R_4 (1:2:2). The seeds sowing depths were: D_1 (0.5 cm), D_2 (1.0 cm), D_3 (1.5 cm) and D_4 (2.0 cm). The measured quantity of water was provided on alternate days in each polybags to keep the soil moist. Germination percentage was recorded daily. Plant height, collar diameter, above and below ground biomass (dry weight) of seedlings was measured after one month of setting of the experiments. SQI was estimated by using formula of Dickson *et al*. [8].

All the experiments were executed using CRD design separately during both the years and the data were statistically analyzed by one way ANOVA [9]. 20 replicates were maintained for each experiment. The mean values of two years for each parameter were presented in tabular form.

RESULTS AND DISCUSSION

(i) Morphological variations and analyses of soil and plant parameters: The morphometric variations in *A. racemosus* at both sites are given in Table 1. The plants at site-I were growing in controlled environment, while that of site-II in open rocky and hilly areas. Flowering, fruiting, seed maturation and dispersal starts nearly 15-20 days before in plants growing at site-I as compared to site-II (Table 1). It grows well in hot and humid condition and does not require irrigation. It needs sandy loamy well drained soil. Data presented in Table 2 clearly revealed that values of total sugars in fruit pulp were higher at site-I as compared to site-II. However, the values of all soil parameters and oil content in seeds were maximum at site-II, because plants are growing in natural dry hilly and rocky habitats.

(ii) Seed morphology and germination behaviour under *in-vitro* conditions: The data on various morphological parameters, *viz*. colour weight, size, volume, density and viability of the seeds collected from different sites are given in Table 3. Studies of seed morphology are considered to be essential for understanding its variability, which is an important adaptation in the life of desert plants. It has an ecological significance for there long term perpetuation in the area as well as introduction to new areas. The seeds are round and black coloured at both sites. The values of all parameters such as fresh & dry weight of fruits and seeds size, weight, volume and density at site- I was higher as compared to site-II.

The data on effect of different growth regulators on various parameters of seeds are given in Table 4. It is evident from this Table that the freshly collected seeds showed 36.00% and 33.00% germination under controlled laboratory conditions at sites-I and II, respectively which can be enhanced to cent percent after pretreatment with 5 and 10 mgl^{-1} concentrations of GA_3. Seed pretreated with 5 mgl^{-1} GA_3 showed cent percent germination at both sites, while 83.00% and cent percent at sites-I and II, respectively with 10 mgl^{-1} GA_3. Maximum R/S ratio was obtained in IBA (5 mgl^{-1}) at site-I. The maximum values for GV and VI was obtained in GA_3 (5 mgl^{-1}) pretreatment at site-II.

The applications of growth regulators have been extensively used for enhancing the growth and development of seedlings under laboratory and nursery conditions. Gibberellins are most prominent growth regulator, which are widely used in cultivated as well as in wild plants. Nadjafi *et al.* [10] observed highest germination percentage in *Teucrium polium* when pretreated with 500-2500 ppm GA_3. Kedia *et al*. [11] reported cent percent germination with maximum values for root and shoot lengths, GV and VI in *Eclipta alba* seeds pretreated with 5 mgl^{-1} as compared to control. Kasera *et al*. [12] observed maximum germination in *Leptadenia reticulata* with 10 and 25 mgl^{-1} of GA_3. Data obtained from present studies clearly reveals that 5 and 10 mg l^{-1} GA_3 pretreatments are suitable for obtaining maximum germination and seedling growth.

(iii) Seed germination behaviour under *in-vivo* conditions: It is evident from Table 5 that cent percent seedlings emerged in D_1 (0.5 cm) depth at both sites. Seeds sown at D_1 depth showed maximum values of SQI at site-I. Among soil ratios experiments, cent percent seedlings emerged in soil having R_2 (1:2:1) ratio at both sites, being maximum values of SQI at site- I. Maximum plant height, collar diameter, shoot and root dry weights were obtained in R_2 (1:2:1) soil ratios at site-I, while at site-II in R_1 (1:1:1) ratios. Kedia *et al*. [13] observed maximum plant height and collar diameter in *E. alba* and *Phyllanthus fraternus* at D_1 depth. Soil is the natural physical and chemical environment of seeds. Sowing depth and soil mixture experiment plays an important role on seed germination, seedling emergence, establishment, vigour and overall plant growth.

Table 1 Morphometric variations of *A. racemosus* collected from two sites-I & II

Characters	Site-I (Cultivated)	Site-II (Wild)
Habitat	Garden	Rocky & hilly
Stem	Whitish grey or brown	Brown
Flowering	Last week of October	First week of November
Fruiting	November	December
Fruit maturation	December to February	January to March
Leaf fall	April	April

Table 2 Variations in parameters of *A. racemosus* collected from two sites-I & II

Parameters	Site-I (Cultivated)	Site-II (Wild)
Total sugars (fruit pulp; mg g^{-1} dry wt.)	27.947	25.853
Seed oil (%)	5.76	9.69
Soil moisture (%)	1.23	1.59
Soil pH	7.22	8.60
Soil EC (mSm^{-1})	0.370	0.450

Table 3 Morphological parameters of fruits and seeds of *A. racemosus* collected from sites-I & II

Sites	Weight of 100 fruits (g)		Seed size (mm)		Weight. of 100 seeds (g)	Volume of 100 seeds (cc)	Density (g cc^{-1})	Viability (%)
	Fresh	Dry	Diameter	Thickness				
Site-I (Cultivated)	9.46 ± 0.45	4.99 ± 0.24	4.03 ±0.29	2.65 ± 0.10	3.70 ± 0.15	2.72 ± 0.03	1.33 ± 0.01	100.00 ± 0.0
Site-II (Wild)	8.64 ± 1.04	4.66 ± 1.45	3.72 ± 0.27	2.62 ± 0.16	3.29 ± 0.16	2.52 ± 0.02	1.21 ± 0.01	96.00 ± 0.0

± = *Standard deviation*

Table 4 Effect of different concentrations of growth regulator pretreatments on various parameters of seed germination and seedlings growth in *A. racemosus* at sites-I & II

Concentrations (mg l^{-1})		Germination (%)		Root length (cm)		Shoot length (cm)		R/S ratio		VI		GV	
		I	II	I	II	I	II	I	II	I	II	I	II
Control		36.00	33.00	1.63	1.32	1.22	1.97	0.127	0.584	246.6	207.5	32.40	27.22
GA_3	5	100.00	100.00	2.69	3.14	3.75	3.66	0.717	0.837	644.0	689.0	202.0	501.1
	10	83.00	100.00	3.54	3.18	3.65	3.56	0.876	0.893	568.5	594.0	137.758	333.3
IAA	5	90.00	85.00	2.92	3.10	3.42	3.50	0.853	0.885	570.6	561.0	202.5	240.8
	10	86.66	80.00	2.76	2.78	3.64	3.72	0.758	0.743	554.6	625.9	184.90	218.2
IBA	5	86.00	60.00	2.93	2.88	3.08	3.40	1.106	0.847	579.6	410.8	147.92	121.2
	10	83.00	62.00	3.16	2.57	3.46	3.32	0.913	0.774	549.4	475.5	137.78	192.2
CD		NS	NS	0.345*	0.0292*	0.108*	0.0376*	0.281*	0.0367*				

*NS = Non-significant; and * = Significant at (P < 0.05) level*

Table 5. Effect of different treatments on various seedling parameters in *A.racemosus* under nursery conditions at sites-I & II

Treatments	Germination (%)		Plant height (cm)		Collar diameter (mm)		Dry weight (g $plant^{-1}$)				SQI	
							Shoot		Root			
	I	II	I	II	I	II	I	II	I	II	I	II
Soil mixture ratios R_1 (1:1:1)	95.0	97.0	15.33	26.33	2.21	2.20	0.047	0.044	0.021	0.124	0.009	0.011
R_2 (1:2:1)	100.0	100.0	21.36	20.00	2.31	2.15	0.094	0.040	0.094	0.085	0.016	0.010
R_3 (2:2:1)	92.0	92.0	15.53	21.43	2.14	1.94	0.040	0.046	0.019	0.048	0.008	0.011
R_4 (1:2:2)	80.0	90.0	16.46	24.63	1.94	2.14	0.074	0.021	0.031	0.015	0.012	0.003
CD	0.88*	0.38*	0.65*	0.55*	0.11*	0.10*	0.003*	0.001*	0.0005*	0.001*	0.001*	0.002*
Sowing depths (cm) D_1 (0.5)	100.0	100.0	20.53	27.56	2.24	2.70	0.082	0.050	0.054	0.123	0.016	0.014
D_2 (1.0)	95.0	94.0	16.70	20.60	2.20	2.56	0.064	0.036	0.034	0.082	0.014	0.009
D_3 (1.5)	87.0	90.0	13.53	24.26	2.14	2.43	0.060	0.032	0.033	0.033	0.013	0.006
D_4 (2.0)	80.0	87.0	11.36	23.30	2.11	2.11	0.059	0.019	0.025	0.085	0.012	0.007
CD	0.78*	0.35*	0.82*	0.81*	0.10*	0.09*	0.008*	0.002*	0.0019*	0.002*	0.001*	0.001*

** = Significant at (P < 0.05) level*

CONCLUSION

Based on the present study, it is concluded that to obtain maximum germination percentage and seedling growth in *A. racemosus* the seeds should be presoaked with 5 and 10 mgl^{-1} GA_3 for 24 h. Seeds sown in D_1 (0.5) depth with R_2 (1:2:1) soil mixture ratio was found to be most favorable for getting optimum seed germination and better seedling emergence under nursery conditions. Cultivated (site-I) is favuorable for obtaining maximum values of all parameters except for oil contents in seeds and soil values for all parameters as compared to wild (site-II) habitats. The more values of oil and soil parameters at site-II are due to plants growing in natural habitats. So for fast multiplication, the germplasm should be collected from cultivated habitat.

Acknowledgements

The authors are grateful to the Professor & Head, Department of Botany, J. N. Vyas University, Jodhpur for providing necessary facilities. Thanks to Dr. M.R.K. Sherwani, Professor of Chemistry, JNV University, Jodhpur for providing laboratory facilities.

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