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Effect of seed priming with plant growth regulators and temperature on *Withania somnifera* L. DUNAL

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Abstract

Seeds of Indian ginseng (Withania somnifera var. Poshita and Jawahar-20) were exposed to various pre-soaking treatments (24, 48 and 72 hours) with different concentrations of three growth regulators (GA_3, IBA, and IAA) ranges from 0.25 to 150 ppm at different temperatures (22, 25 and 28^{0}C) to observe the combined effect of these factors on germination. Exposure at 28^{0}C with 100ppm GA_3 for 24 hours soaking time and 125 ppm GA_3 with 72 hours soaking time showed most synergistic effect on germination in J20 (76.66%) and Poshita (73.33%), respectively. Though IBA and IAA also showed significant increase in germination in both the varieties but it was not as effective as GA_3. Study concluded that growth regulators and appropriate temperatures play a synergistic role in breaking the seed dormancy by influencing various metabolic activities at this moment.

INTRODUCTION

Medicinal plants are integral components of pharmaceutical industries as suppliers of basic raw materials for medicines, perfumes, flavours and cosmatics etc. They serve as valuable source of income owing to their export potential. For successful cultivation of these crops, knowledge on their propagation techniques has great bearing on realizing a good harvest. A good quality seed without dormancy is very essential for sowing to get high germination percentage, and better establishment of crop. The germination capacity of seeds of medicinal plants is normally low due to the accumulation of dormancy causing metabolites in the seeds [12].

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Withania somnifera (L.) Dunal.) is one such medicinal herbs. It is 50-150cm tall erect self pollinating shrub, has a great demand in pharmaceutical industries. All the parts of this plant specially roots are used in ayurvedic and unani medicines. It is prescribed for various diseases like hiccup, bronchitis, dropsy, rheumatism and female disorders. It has antibiotic, antiviral, antiamoebic, antiarthritic and anti-inflammatory properties and a general tonic for overall health. [14, 16]. Various active withanolides have so far been isolated from *Withania somnifera* and reported to possess immune modulatory effects, anti stress and hepato-protective roles in human practices [4].

Very small seed size and hard seed coat of *Withania somnifera* creates dormancy and farmers face problems in germination. To break dormancy a variety of methods are in vogue

worldwide these days. Of all different methods of breaking dormancy, priming of seeds with certain chemicals including different types of plant growth regulators (PGRs) and appropriate temperatures are contemplated as the most effective factors. Gibberellic acid (GA_3) is the most widely used plant growth regulator (PGR) to improve seed germination in different plant species [3, 15]. Application of GA_3 enhanced growth of plants by increased cell wall plasticity, which leads to breaking of starch into simple sugars. These sugars, in fact, cause reduced cell osmotic potential which results in absorption of high amount of water, ultimately results in cell elongation and growth [1]. Despite GA_3 various other enhancing PGRs such as IBA, IAA, NPA and 2,4 D are also used for soaking seed for germination at low concentrations. These effects are subjected to variation depending on form and species of plants. Keeping these views in mind we planned a combined experiment of seed priming with various soaking time of PGRs and temperature to observe the effect on germination in two varieties of *W. somnifera* i.e., cv. Poshita and cv. J 20.

MATERIALS AND METHODS

The seeds of *Withania somnifera* (varieties- Poshita and Jawahar-20) were obtained from Medicinal Aromatic Plants Research and Development Center (MRDC), G.B. Pant University of Agriculture and Technology, Pantnagar and Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow.

Seed viability assessment

A seed viability test was conducted to ensure that seeds were viable. The seeds were imbibed for 24h in water, cut along the margin without damaging the embryo and soaked in colorless 0.1 % solution of 2,3,5- triphenyltetrazolium chloride (TTC) for 1 h at 25^{0}C in dark**.** Seeds were removed from TTC solution and washed with distilled water. The seeds were then viewed under light microscope to observe the stained embryos. Whole embryos of viable seeds appeared bright red in color [13].

Soaking of seeds with plant growth regulators and germination studies:

After seed viability test forty seeds were soaked in different concentrations of hormone (GA_3, IBA, and IAA) in the ranges from 25 to 150ppm for 24, 48 and 72 h at room temperature and kept for germination in petri plates which were lined by moist filter paper and incubated in a growth chamber at 22, 25 and 28°C. Percent germination was recorded as number of seeds germinated over 15 days. The seeds were examined daily till completion of germination.

RESULTS

Germination in the seeds of *W somnifera* is a complex process as they are too small and having hard seed coat. Low viability of seeds also concern with these factor. Long period between harvesting and trials may further cause the reduction in viability. Hence, it is necessary to check the viability of seeds before performing the experiment. The tetrazolium is very reliable for this purpose. A high viability percentage i.e., 79.99% in J 20 and 70% in Poshita was obtained in the present study.

As the growth regulators priming is an important phenomenon to overcome the dormancy, various growth regulators with three soaking durations (24,48,& 72 hours) in three temperature regimes (22,24 & 28 ^{0}C) were tested. The viable seeds of *Withania somnifera* (var. J 20 & Poshita) were treated with Gibberellic acid (GA_3), Indole 3-butyric acid (IBA) and Indole 3-acetic acid (IAA) with concentration ranging from 25- 150 ppm. The results indicated that hormone type, time of priming, hormone concentration and temperature regimes had significant effect in seed germination. Out of three growth regulators, gibberellic acid (GA_3) treatment was most effective in enhancing the germination. The maximum germination was found in Jawahar-20 i.e., 76.66%, when the seeds were soaked for 24 hours with 100ppm GA_3 at 28 ^{0}C, it was a tremendous increase of about eleven fold, however a significant increase in germination was found in all the treatments with all temperature regimes, showing a synergistic effect with temperature ranges between 22 -28 ^{0}C. Soaking

time of 48 h and 72 h was least effective in comparison to 24 h at final germination.In variety Poshita results were slight different., 125ppm GA_3 with 72 h soaking time at temperature regime 28 ^{0}C was most effective. We found 73.33% germination n this combinations of PGR and temperature however other soaking treatments of plant growth regulators with different incubation temperatures to the seeds gave significant percent germination (Table 1).

The exogenous application of IBA and IAA also showed significant increase in germination of both the varieties but they were not as good as GA_3. Moreover, 100ppm IBA as well as IAA gave better response in comparison to other concentrations with 63.33% germination. 100ppm IBA with 24 hours soaking time and priming of 28 ^{0}C was more effective in Jawahar- 20 than other treatments. However, 22 ^{0}C temperature and 48 hours soaking of IBA was effective for variety Poshita. No germination was found at 28 ^{0}C, it means it did not show any compatibility with priming of IBA (Table 2 & 3). Again IAA showed zero results at 28^{0}C for Jawahar-20 and at 25^{0}C & 28^{0}C for Poshita however, 100ppm IAA with 25^{0}C was effective for germination (53.33%) in Jawahar-20 and same conc. with 22^0 C was effective (56.67%) for Poshita variety.

DISCUSSION

Tetrazolium chloride test has great concern with germination tests. It revealed 79.99% viability in Jawahar 20 and 70 % in Poshita. This high percentage of viability is probably due to the short period between the harvesting and the trials of the seed. It was reported in a study that the viability of seeds of *W. somnifera* was decrease with the increasing duration between harvesting and experimental trials [17].Therefore, to obtain maximum germination results seeds of current season are recommended [9].

Seed priming caused physiological and biochemical changes before germination. Various seed priming technique have been developed including hydropriming, osmopriming, halopriming, biopriming, Potassium salt and hormone priming and thermopriming etc. Each priming technique has its varying effects on germination depending upon plant species, stage of development, priming agent and soaking time. In present investigation a synergistic effect of hormone priming and thermopriming was observed. Like plant growth regulators appropriate temperature at the time of germination is very important, exposure of high temperature can reduce the germination as well as seedling growth in most of the cases however

Table 1. Effect of soaking periods of GA_3 and temperature on seed germination of *W. somnifera* varities Jawahar- 20 and Poshita

GA_3 (ppm)	Soaking Period (hr.)																	
	Jawahar 20									Poshita								
	24			48			72			24			48			72		
	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C
Control	13.33*	16.67	6.67	13.33	13.33	3.33	10.00	10.00	6.66	10.00	10.00	16.67	13.33	6.67	16.67	6.67	10.00	23.33
25	26.67	23.33	16.67	20.00	26.67	13.33	20.00	16.67	10.00	16.67	13.33	26.67	16.67	16.67	13.33	13.33	13.33	26.67
50	30.00	30.00	20.00	26.67	30.00	16.66	23.33	23.33	16.66	20.00	23.33	33.33	23.33	33.33	23.33	16.67	16.67	30.00
75	36.67	33.33	23.33	33.33	36.67	23.33	26.67	30.00	23.33	40.00	30.00	40.00	36.67	36.67	30.00	23.33	23.33	36.67
100	63.33	56.67	**76.66**	53.33	46.67	43.33	43.33	66.67	33.33	53.33	60.00	63.33	63.33	56.67	33.33	43.33	56.67	46.67
125	56.67	36.67	43.33	46.67	40.0	46.66	36.67	36.67	40.00	36.67	43.33	40.00	43.33	33.33	50.00	30.00	43.33	**73.33**
150	43.33	43.33	33.33	40.00	40.00	63.33	30.00	40.00	56.66	30.00	33.33	36.67	30.00	26.67	36.67	23.33	23.33	56.67
SEM	1.475	0.667	0.8637	1.120	0.630	0.701	0.984	0.488	0.534	0.756	0.766	0.926	0.826	0.713	1.000	0.690	0.873	0.959
CD at 5%	4.472	2.022	2.6196	3.396	1.911	2.127	2.984	1.480	1.621	2.293	2.324	2.808	2.506	2.162	3.033	2.093	2.647	2.910

* per cent germination

Table 2. Effect of soaking periods of IBA and temperature on seed germination of *W. somnifera* varities Jawahar- 20 and Poshita

IBA (ppm)	Soaking Period																	
	Jawahar 20									Poshita								
	24 h			48 h			72 h			24 h			48 h			72 h		
	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C
Control	10.00*	10.00	10.00	13.33	13.33	3.33	6.67	6.67	3.33	10.00	13.33	-	13.33	13.33	-	6.67	3.33	-
25	16.67	16.67	13.33	16.67	20.00	6.67	13.33	13.33	10.11	16.67	16.67	-	16.67	20.00	-	13.33	13.33	-
50	23.33	30.00	16.67	20.00	26.67	10.00	20.00	23.33	13.33	20.00	20.00	-	23.33	26.67	-	16.67	16.67	-
75	30.00	36.67	23.33	26.67	30.00	16.67	23.33	26.67	16.67	40.00	26.67	-	36.67	30.00	-	23.33	20.00	-
100	60.00	53.33	**63.33**	40.00	43.33	26.67	50.00	43.33	23.33	53.33	43.33	-	**63.33**	46.67	-	43.33	40.00	-
125	40.00	26.67	33.33	23.33	30.00	46.67	30.00	33.33	33.33	36.67	36.67	-	43.33	36.67	-	30.00	26.67	-
150	36.67	20.00	6.67	16.67	26.67	33.33	26.67	20.00	23.33	30.00	23.33	-	30.00	26.67	-	23.33	23.33	-
SEM	0.951	0.519	0.418	1.175	0.454	0.218	0.934	0.333	0.398	0.756	0.909	-	0.826	0.900	-	0.690	0.701	-
CD at 5%	2.885	1.575	1.267	3.564	1.378	0.662	2.834	1.011	1.208	2.293	2.755	-	2.506	2.729	-	2.093	2.127	-

* per cent germination

medium temperature enhanced the germination as various enzymes required for breakdown of complex stored material are not destroyed high temperature.

In our investigation $GA_{3,}$ IBA and IAA with different priming duration and different temperatures had significant effect on germination in both the varieties 'Jawahar- 20' and 'Poshita'. Seeds with various growth regulators priming improved the percent germination and reduced the mean germination time although the germination was started after 5 days of sowing and continued up to 15 days in different treatments of growth regulators as well as in control with varying germination percentage, it might be due to altered physiology of embryos and liberating enzymes in treated seeds so that the development process occurs more rapidly after priming [8]. GA_3 stimulate germination by inducing hydrolytic enzymes that break the barriers such as endosperm and seed coat. Seeds of *W. somnifera* have very small and hard seed coat which creates dormancy, so quality of seed and optimal physiological conditions are of prime importance [11]. The role of GA_3 in breaking of dormancy has been documented by different workers [19, 7]. An interaction between endogenous and exogenous levels of hormones also might have play important role in germination

Table 3. Effect of soaking periods of IAA and temperature on seed germination of *W. somnifera* varities Jawahar- 20 and Poshita

IAA (ppm)	Soaking Period																	
	Jawahar 20									Poshita								
	24 h			48 h			72 h			24 h			48 h			72 h		
	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C	22^{0}C	25^{0}C	28^{0}C
Control	13.33*	10.00	-	6.67	13.33	-	10.00	10.00	-	10.00	-	-	10.00	-	-	10.00	-	-
25	16.67	13.33	-	13.33	20.00	-	13.33	23.33	-	13.33	-	-	13.33	-	-	20.00	-	-
50	20.00	20.00	-	23.33	33.33	-	23.33	26.67	-	23.33	-	-	16.67	-	-	23.33	-	-
75	33.33	23.33	-	26.67	40.00	-	30.00	40.00	-	26.67	-	-	20.00	-	-	30.00	-	-
100	46.67	33.33	-	36.67	53.33	-	40.00	**63.33**	-	43.33	-	-	**56.67**	-	-	46.67	-	-
125	30.00	16.67	-	23.33	40.00	-	20.00	43.33	-	30.00	-	-	26.67	-	-	33.33	-	-
150	23.33	13.33	-	13.33	36.67	-	13.33	36.67	-	10.00	-	-	16.67	-	-	26.67	-	-
SEM	0.787	0.630	-	0.816	0.504	-	0.735	0.378	-	0.766	-	-	0.504	-	-	0.943	-	-
CD at 5%	2.386	1.911	-	2.476	1.528	-	2.228	1.146	-	2.324	-	-	1.528	-	-	2.859	-	-

* per cent germination

and plant growth. It was also reported that GA_3 enhance the germination of seeds exhibiting physiological, morphological or morpho physiological dormancy [6].

In case of *W somnifera,* it was observed that of the growth regulator priming with GA3, IBA and IAA alone not overcame the problem of seed dormancy. Thermopriming with different temperatures gave excellent response during germination.Maximum germination was observed in Jawahar -20 as compared to Poshita under the treatment of GA_3. This could be attributed that poshita seeds were more dormant than that of Jawahar-20. Dormancy may be due to the presence of some inhibitors, hard seed coat, low internal hormone or underdeveloped embryos. There are various reports on the application of gibberellic acid in alleviating innate and environment-induced dormancy [18]. By providing long soaking period of growth regulators we can improve the germination percentage. IAA and IBA induce cellular elongation, cell division and emergence of radical across the endosperm and seed coat [2]. IAA and IBA also give a better result as far as the seed germination is concern in both the varieties but they were not performing well as GA_3.

It means a synergistic effect of hormonal concentration and appropriate temperature is required for better results of germination. In a previous study it was suggested, that various mechanisms prevent germination when temperature raised than normal and followed by possible very cold nights. In another study seeds of *W. somnifera* also did not germinate when exposed to constant temperatures in continuous darkness [5].

CONCLUSION

Dormancy is common severe problem in medicinal plants and can be overcome by various strategies such as scarification, stratification, hot water treatment and acid treatment etc. Seed priming is also one of these techniques for breaking seed dormancy. In present study the seeds of *Withania somnifera* (varieties Jawahar-20 & Poshita) were primed by various combinations of plant growth regulators and different temperature regimes. All the plant growth regulators at 28^0C temperature showed synergistic effect on germination of seeds. Among IAA, IBA and GA_3, GA_3 was most effective than others for germination enhancement in both the varieties. However, Poshita was more dormant than J20 as it require more soaking period. The study also revealed that gibberellic acid improved seed germination in *W.somnifera* when compared with other growth regulators.

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