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RESEARCH PAPER

Methods to Break Seed Dormancy of *Andrographis paniculata* (Burm.f.Nees): An Important Medicinal Herb of Tropical Asia

[1]Rita N. Kumar, Sudeshna Chakraborty and [2]**Nirmal Kumar J.I**
[1]Department of Biological and Environmental Sciences,
N. V. Patel College of Pure and Applied Sciences
Vallabh Vidyanagar-388120, Gujarat, India
E-mail:- nfils09@gmail.com
[2]P.G Department of Environmental Science and Technology
Institute of Science and Technology for Advanced studies and Research (ISTAR),
Vallabh Vidyanagar-388120, Gujarat, India

ABSTRACT

The aim of the present study was to enhance the germination rate of Andrographis paniculata seeds which have a very low germination rate under normal conditions. The seeds were soaked in different hormonal solutions i.e. GA (10ppm), IAA (10ppm), IBA (10ppm), Kinetin (10ppm), GA+ Kintetin (10 ppm), IAA+ Kintetin (10ppm) and IBA + Kintetin (10 ppm), and 50°C hot water at three treatment times (5,10 and 15 min) before placing in Petri plates. The seeds of A. paniculata treated in hot water for 5 min showed maximum germination percentage of 93%. Analysis of variance indicated that both hormonal and hot water treatments had a significant effect on seed germination and final germination percentage. The results showed that hormonal treatments are not useful methods for breaking the seeds dormancy.

KEY WORDS: *Andrographis paniculata, germination, Gibberellic acid, Indole acetic acid, Indole butyric acid, Kintein, Hot water*

INTRODUCTION

Andrographis paniculata (Burm.f.Nees), a well known herb belonging to the family Acanthaceae is grown widely in tropical areas of Asia like India, Pakistan and Sri Lanka and commonly known as "Kalmegh". It is known as the "King of Bitters [1]. Numerous pharmacological activities of the *A. paniculata* extract have been reported including antimicrobial, anticoagulant, antifertility, anthelmintic, hyposensitive, antioxidant, anti-inflammatory and antiplasmodic activities [2, 3,4].The medicinal property of the plant is mainly because of presence of andrographolide (an active diterpene lactone) [5,6,7]. Inspite of being medicinally important the major problem in the propagation of *A.paniculata* is related to seed germination. It is therefore necessary to find out adequate solutions to overcome seed dormancy.

To accelerate breaking of seed dormancy, hormones have been applied in several studies [8]. Plant growth regulators such as GA, IAA, IBA, Kintein and mechanical scarification such as hot water have been recommended to break dormancy and enhance germination [9]. Therefore, the objective of the present investigation was to determine the efficacy of various scarification treatments on seed germination pattern of *A. paniculata*.

MATERIALS AND METHODS

Seed Collection

Mature pods of *A. paniculata* were collected using Random Sampling Technique (RST) from the plants grown in experimental plots in Botanical Garden of Charuter Vidyamandal (CVM) in the month of November-December 2008. After dehiscence of the fruits in the laboratory conditions, equal samples of seeds were combined to give one bulk population sample from which sub samples were taken for germination test. A completely randomized design with three replications was used, while the dependent variables measured were 3, 6,9,12 and 15 days periods of germination.

Seed germination and growth conditions

Washed seeds of *A. paniculata* were surface sterilized with 0.1 %(w/v) mercuric chloride. Seeds were thoroughly rinsed with double distilled water prior to applying any treatments. The treatments were as follows:

Hormone treatment

GA, IAA, IBA, Kinetin, were mixed with distilled water and made to different combinations. The seeds were soaked in seven different treatments of hormones i.e. GA (10ppm), IAA (10ppm), IBA (10ppm), Kinetin (10ppm), GA+ Kintetin (10 ppm), IAA
+ Kintetin (10ppm) and IBA + Kintetin (10 ppm) at room temperature for 15 minutes and then were inoculated in the room temperature.

Mechanical Stratification

In the mechanical stratification treatment, seeds were treated at 50^{o}C hot water for three treatment time (5, 10 and 15 min).

The seeds were also soaked in sterile distilled water and then kept for germination which is kept as control. Germination experiments were conducted using three replications of 15 seeds per treatment. Seeds were placed on single layered sterile Whatman No. 1 filter paper moistened with 5 ml of distilled water in sterilized petriplates of 15cm diameter.

Germination

The cumulative percentage germination (CPG) and mean germination time (MGT) was calculated using the method of Youngsheng and Sziklai [10]. The germination value (GV) was computed following the method of Djaranshir and Pourbeik [11].

Statistical Analysis

Data were statistically analyzed by using computer software Microsoft Excel and SPSS ver. 12.0 to explore possible treatment variations. The Analysis of Variance (ANOVA) and Duncan's multiple range test (DMRT) were also used for the analysis.

RESULTS

During present investigation it was observed that the hot water favored seed germination then hormonal treatment.

Hormone treatments

The germination percentage of the seeds depends on the type of treatment and a significant difference in germination was observed in the seeds treated with various hormonal treatments i.e. GA (10ppm), IAA (10ppm), IBA (10ppm), Kinetin (10ppm), GA+ Kintetin (10 ppm), IAA+ Kintetin (10ppm) and IBA + Kintetin (10 ppm). As a whole, different hormonal treatments conspicuously increased germination rate, germination value and germination percentage. Maximum germination i.e. 93.3% was recorded in the seed treated with GA (10 ppm) which was followed by GA+ Kintetin (10 ppm), IAA+ Kintetin (10ppm) and IBA + Kintetin (10 ppm) which showed germination percent of 53.3%. (Table 1).

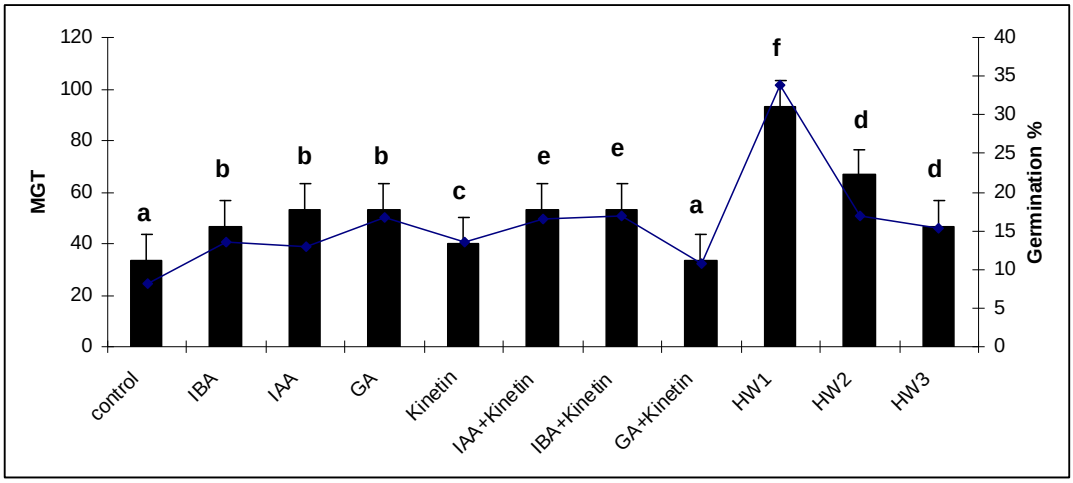
Mechanical Stratification

The results of the ANOVA showed that hot water treatment had a significant impact ($P<0.05$) on the examined germination. Application of hot water (50^{o}C) for 5 min gave 93.3% germination. The minimum germination percentage was recorded in the seeds treated with hot water treated for 10 and 15 min which showed a germination percentage of 66.6% and 46.6% respectively (Fig 1).

DISCUSSION

Hot water treatments have been reported to enhance germination of hard coated seeds by elevating water and O_2 permeability of the testa [12]. Muhammad and Amusa [13] studied the germination percentage of *Tamarindus indicus* where the germination percentage and mean germination time increased by 50% after treating with hot water for 5 min. The treatment time exerts significant effect on seed germination [14]. Duguma et al. [15] observed higher germination percentage in the seeds treated with hot water for 5 min in *Leucaenia leucocephala* and *Acacia nilotica*. Similar results were recorded for *Atropa bella- donna* seeds by Genova et al. [16] where the dry matter content and -

Fig 1:- Correlation between Mean Germination time (MGT) with Germination %



Mean values by the same letter are not significantly different at the 0.05 level according to the Duncan test.

Table: - 1 Effects of Hormonal and Hot water treatments on Germination Value, Cumulative % Mean germination.

Treatment	Days of Germination														
	3			6			9			12			15		
	M	CMG	GV	M	CMG	GV	M	CMG	GV	M	CMG	GV	M	CMG	GV
Hormone															
Control	0	0	5	1	7	5	3	20	5	4	27	5	5	33	5
IBA (10 ppm)	0	0	10	2	13	10	3	20	10	5	33	10	7	17	10
IAA (10 ppm)	0	0	10	2	13	10	3	20	10	3	20	10	8	53	10
GA (10 ppm)	1	7	18	4	27	18	5	53	18	5	53	18	8	53	18
Kinetin (10 ppm)	0	0	9	3	20	9	4	27	9	5	33	9	6	40	9
IAA+ Kinetin (10 ppm)	0	0	15	4	27	15	5	33	15	5	33	15	8	53	15
IBA+ Kinetin (10 ppm)	0	0	15	3	20	15	5	33	15	6	40	15	8	53	15
GA+ Kinetin (10 ppm)	0	0	6	2	13	6	3	20	6	4	27	6	5	33	6
Hot water															
Control	0	0	5	1	7	5	3	20	5	4	27	5	5	33	5
50°C (5 min)	4	27	71	8	54	71	9	60	71	13	87	71	14	93	71
50°C (10 min)	1	7	18	1	7	18	4	27	18	5	33	18	10	67	18
50°C (15 min)	1	7	13	1	7	13	5	33	13	6	40	13	7	47	13

germination percentage increased after treating them with hot water for 5 minutes. Improvement in the germination was recorded by Sundraraj et al. [17] in *Tephrosia purpurea* by scarification with sand followed by pre soaking in hot water at 50°C for five minutes. Treating seeds in hot water for more

time could affect the seeds adversely by decreasing the viability of the seeds [18]. Endogenous gibberellins have been widely studied in relation to the breaking of seed dormancy in various species. GA has been exogenously applied as a substitute for stratification and has increased germinations in many plant species, including *Leucospermum*, *Fagus sylvatica* and *Helianthus* and many times it is applied as a substitute for scarification [19, 20, 21]. Some of the studies also showed that the results of exogenous applications of GA on the breaking of seed dormancy and seed germination can be differed widely among species and within species [22]. It has been reported that treatment with GA can increase the formation of rough endoplasmic reticulum and polyribosmes. Moreover, it has been found that GA stimulates the synthesis of m RNA which is specific for α amylase [23]. With respect to the germination pretreatments, our results demonstrate that hormone treatments applied to *A. paniculata* is not showing any significant germination percentage compared to hot water treated seeds. The seed coat plays an important role in the present species, as it prevents water uptake. The strong inhibitory effect of the seed coat on seed germination may be caused by several possible mechanisms, including mechanical constraints, prevention of water and oxygen uptake and retention of production of chemical inhibitors [24]. The integument breaking or softening, for instance, is needed to remove dormancy imposed by seed coat hardness or impermeability [8].

From the present study it is revealed that the low germination percentage of *A. paniculata* is due to hard seed coat. Hence the hot water treatment for 5 min increased the germination by softening the hard seed coat layer.

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