Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 114 (2018) 249-255 EISSN 2392-2192

SHORT COMMUNICATION

Chemical-induced seed germination and enhancement of seed potential of seven wild plant taxa of Ericaceae in India

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ABSTRACT

Pretreatment of seeds of seven wild plant taxa (*viz*., *Gaultheria hookeri* C. B. Clarke, *G. stapfiana* Airy Shaw, *G. semi-infera* (C. B. Clarke) Airy Shaw, *G. trichophylla* Royle var. *ovata* Panda & Sanjappa, *Lyonia ovalifolia* (Wall.) Drude var. *ovalifolia*, *Pieris formosa* (Wall.) D. Don and *Vaccinium glauco-album* C. B. Clarke) in the family Ericaceae using Na-dikegulac (Na-DK) for 8 hours (4 + 4 h in two installments) before keeping in ambient storage condition (32±2 ºC) for different durations (0 and

(Received 04 November 2018; Accepted 20 November 2018; Date of Publication 27 November 2018)

20 days) slowed down the rapid loss of germination and reduced the time (h) required for 50% seed germination (T_{50}). Concomitantly, the reduction of protein level as well as the activity of catalase of seed kernels during storage period was ameliorated to a significant extent in the chemical pretreated seed lots.

***Keywords*:** Seed germination, seed metabolism, T_{50} of germination, protein, catalase

1. INTRODUCTION

Deterioration of seeds is a natural catabolic process which results in serious impairment of seed viability and consequent termination of life span. This process may be accelerated by some pathogenic attack or by adverse environmental conditions. Maintenance of vigour and viability of seeds in tropical countries like India is a matter of serious concern to the crop growers because of high temperature and high relative humidity (RH) prevailing in major parts of the country almost throughout the year. These two environmental factors strongly impair seed and seedling health and cause to reduce percent seed germinability and seedling performance at a rapid rate (Copeland and McDonald, 1995; Desai *et al.,* 1997; Maity *et al*., 2000; Bhattacharjee, 2001).

Thus, Indian cultivators are very often compelled to use low vigour seeds in agriculture. To get rid of this problem, strategies are now being undertaken to improve the storage potential of seeds for enhancing their life span (Pathak and Basu, 1980; Chhetri *et al.*, 1993; Basu, 1994; Aditya et al. 2014). Being the experimental seeds are grown in temperate climate and in higher altitudes, these are often unable to germinate in the plane land agroclimatic condition. Keeping this problem in mind, an attempt is made in this investigation to enhance the seed germination and metabolism of seven wild plant taxa in the family Ericaceae using Na-dikegulac (Na-DK). Experiments of this investigation were carried out under ambient storage condition (32±2 ºC) condition to obtain more or less uniform and expeditious results. Thus, the objective of this investigation is to explore the efficacy of Na-Dk and metabolism on enhancing percentage of seed germination of a few wild plant taxa under ambient storage condition by analysing germination behaviour and metabolic status of seeds.

2. MATERIALS AND METHODS

Experiments of the present investigation were carried out with freshly collected seeds of seven wild plant taxa (*viz*., *Gaultheria hookeri* C. B. Clarke, *G. stapfiana* Airy shaw, *G. semi-infera* (C. B. Clarke) Airy Shaw, *G. trichophylla* Royle var. *ovata* Panda & Sanjappa, *Lyonia ovalifolia* (Wall.) Drude var. *ovalifolia*, *Pieris formosa* (Wall.) D. Don and *Vaccinium glauco-album* C. B. Clarke) in the family Ericaceae from Sikkim Himalaya at altitudes ranging from 2500 – 4000 m. Seven taxa are correctly identified at Central National Herbarium (CAL).

After surface sterilization (0.1% $HgCl_2$ for 90 seconds) the seed samples were separately pre-soaked in the aqueous solution of Na-dikegulac (Na-Dk, 100 µg/ml) for 4 hours and then dried back to the original dry weight of the seeds. This was repeated twice allowing maximum penetration of the chemicals present in the aqueous solution.

The pretreated seed lots were taken in separate cloth bags and kept under alternating freezing (4 hours) followed by thawing (32±2 ºC for 4 hours) in two installments. This experimental set up was kept at 32±2 ºC for 20 days allowing the seeds to experience natural ageing treatment. Data were recorded after zero (0) and 20 days. To analyse the percentage germination, four groups of 100 seeds *i.e.* 400 seeds of each treatment were transferred to separate Petri dishes containing filter paper moistened with 10 ml distilled water. Germination data were recorded after 96 hours of seed soaking following the International Rules for Seed Testing (ISTA, 1976). The time for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al*. (1984).

Protein contents as well as the activity of catalase enzyme was analysed from seed kernels of each sample. Protein level was estimated as per the methods of Lowry *et al.* (1951). Extraction and estimation of the enzyme catalase was made following the method of Snell and Snell (1971) as modified by Biswas and Choudhuri (1978). For assaying these enzymes, the blank was taken as zero time control and the activity was expressed as $(\Delta OD \times T_V)/(t \times v)$, where ΔOD is the difference of OD of the blank and sample. T_V is the total volume of filtrate, t is the time (min) of incubation with the substrate and v is the volume of filtrate taken for incubation (Fick and Qualset, 1975). Data were statistically analysed at the treatment and replication levels and least significant difference (LSD) values were calculated at 95% confidence limits (Panse and Sukhatme, 1967).

3. RESULTS

Data clearly revealed that under accelerated ageing condition percentage seed germination was significantly decreased both in control and treated seed lots. But the magnitude of decrease was found to be much less when seeds were pretreated with aqueous solution of Na-dikegulac. The seed pretreating agent also significantly reduced the time required for 50% germination (T_{50}) of seeds (Table 1). This effect was found remarkable at later observation periods. The level of protein (Table 2) experimental seeds were remarkably reduced in control samples than the treated ones. The activities of the enzyme catalase (Table 2) was found to decline with ageing process and the declining trend was arrested by the seed pretreateating agent.

4. DISCUSSION

The results of the present study show that during ambient storage the ageing and deterioration of experimental seeds as would be evident from the progressive fall of germination percentage and higher T_{50} hours (Table 1). Pretreatment of the seeds with Na-DK significantly alleviated the loss of germination and reduced T_{50} hours (Table 1), alleviated the loss of protein (Table 2) as well as catalase (Table 2) enzyme.

The proposal that a decrease in membrane lesions might play a significant role in deterioration of seeds has been supported by the work on solute leakage accompanying a loss in germinability and viability (Ching and Schoolcraft, 1968, Harmann and Granett, 1972, Powell and Matthews, 1977). The ability of seeds to reorganize its membrane rapidly as the desiccated tissue rehydrates is a crucial factor for successful germination and this is clearly

documented in the literature (Simon, 1974). Much evidence has been put forward to suggest that membrane status within the germinating embryo is an important factor in deterioration (Harmann and Mattick, 1976; Ponnachan *et al.*, 1993; Desai *et al.*, 1997; Kamalakkannan and Stanely, 2003; Mishra *et al.*, 2004a; Pati & Bhattacharjee 2015). Thus, in the present study, the concomitant reduction of seed germinability is the indicative of damage of seed membrane and consequent loss of seed vigour and viability. The chemical-induced substantial amelioration of all these deleterious effects are indicative of seed potentiation under adverse storage environment. Efficacy of the Na-DK on the maintenance of seed can also be supported from the data on biochemical analysis of seeds kept at ambient storage for 20 days. In the assayed seeds experimental chemical helped to check the decline of protein along with catalase.

The results therefore point out that although deterioration is a common phenomenon in treated and control sample of the seed species, the catabolic processes within the treated seed samples remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environment of plane lands. Available reports show that during seed storage a loss of some vital cellular components including protein, carbohydrates, nucleic acids were also occurred (Kole and Gupta, 1982; Bhattacharjee and Gupta, 1985). Catalase is regarded as a scavenger enzyme (Fridovich, 1976) and higher activity of this enzyme is indicative of higher plant vigour (Sarkar and Choudhuri, 1980; Pati and Bhattacharjee, 2003; Pati & Bhattacharjee 2011 & 2012).

Table 1. Effect of seed pretreatment with Na-dikegulac (Na-DK;100 µg/ml) on percentage seed germination and T50 of seed germination of seven wild plant taxa in Ericaceae from Sikkim Himalaya.

Seeds were presoaked with the chemical or distilled water for 4h and then dried back to original seed weight. This was repeated twice. Pretreated seed samples were kept under ambient storage condition (32 ± 2 ºC) and data were recorded after zero (0) and 30 days.

Different seed samples	Percentage seed generation				T50 of seed germination			
	Days after analyses							
	0		30		0		30	
	Control	Na-DK	Control	Na-DK	Control	Na-DK	Control	Na-DK
Gaultheria hookeri	15.00	28.00	14.0	20.00	NA	NA	NA	NA
G. stapfiana	20.00	30.00	11.00	20.00	NA	NA	NA	NA
G. semi-infera	40.00	56.00	30.00	50.00	NA	78.00	NA	84.00
G. trichophylla var. *ovata*	16.00	26.00	09.00	15.00	NA	NA	NA	NA
Lyonia ovalifolia var. *ovalifolia*	41.00	54.00	30.00	51.00	NA	72.00	NA	78.00
Pieris formosa	43.00	55.00	32.00	50.00	NA	66.00	NA	72.00
Vaccinium glauco-album	40.00	50.00	26.00	48.00	NA	78.00	NA	NA
LSD (p = 0.05)	0.92	1.12	0.32	1.09	NC	5.17	NC	5.08

NC: Not calculated; NA: Non attainment of 50 % germination of seeds

Table 2. Effect of seed pretreatment with Na-dikegulac (Na-DK;100 µg/ml) on protein (mg/g fr. wt.) and catalase (ΔODxTv/txv) of seven wild plant taxa in Ericaceae from Sikkim Himalaya.

Different seed samples	Protein				Catalase			
	Days after analyses							
	0		30		0		30	
	Control	Na-DK	Control	Na-DK	Control	Na-DK	Control	Na-DK
Gaultheria hookeri	184.00	196.24	100.01	140.01	65.04	74.01	40.01	58.07
G. stapfiana	127.00	138.00	98.90	102.00	50.91	60.13	29.03	40.97
G. semi-infera	178.00	190.00	101.97	132.03	61.00	70.20	30.37	44.98
G. trichophylla var. *ovata*	168.04	180.01	98.88	194.00	58.88	68.28	29.00	42.03
Lyonia ovalifolia var. *ovalifolia*	100.28	112.39	79.07	98.00	44.13	50.01	23.07	38.88
Pieris formosa	163.89	172.92	84.04	102.98	51.07	60.18	28.98	40.01
Vaccinium glauco-album	174.00	188.01	93.06	113.09	52.78	62.25	29.18	41.05
LSD (p = 0.05)	10.04	11.08	8.27	8.38	4.04	4.78	1.93	2.24

Treatments and recording of data as in Table 1.

5. CONCLUSIONS

In this investigation, the chemical-induced arrestation of rapid loss of the enzyme activity was indicative of strengthening the defence mechanism by the chemical under adverse storage condition. It can be concluded from the results of this investigation that Na-DK is effective in enhancing storage potential of experimental seeds. Thus, invigouration property of the present seed pretreating agent seems to be apparent from our experimental results.

Acknowledgements

We are grateful to Prof. N. K. Verma, Ex-Head, Department of Botany and Forestry, Vidyasagar University for providing all the facilities to conduct this investigation, to Professor (Dr.) A. Bhattacharjee, Plant Physiology & Biochemistry Laboratory, The University of Burdwan for manuscript correction and to Dr. M. Sanjappa, Director, Botanical Survey of India for providing necessary help during field studies in Sikkim Himalaya on 3rd, 4th, 5th and 6th October, 2007.

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