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EFFECT OF SOME CHEMICAL TREATMENTS ON SEED GERMINATION AND DORMANCY BREAKING IN AN IMPORTANT MEDICINAL PLANT *OCHRADENUS ARABICUS* CHAUDHARY, HILL C. & A.G. MILL.

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Abstract

The seeds of *Ochradenus arabicus*, a medicinally important plant endemic to Saudi Arabia, become dormant and thus do not germinate easily. An attempt was made to improve seed germination and break the dormancy. The seeds were subjected to 2 plant growth regulators [GA_3 (25-500 µM) and BAP (25-350 µM)] and two chemicals (KNO_3 and thiourea) at 0.1-0.5% concentrations. Germination of seed was recorded after 20 days of sowing the seed in different treatments. Maximum germination was obtained by GA_3 at 100µM, however, decline in germination was observed at higher concentrations of GA_3. The germination of seeds was found to be improved upon storage for 6 and 12 months. In general, all treatments showed increased germination compared to that of control. This might have occurred due to the slow release of an inhibitory dormancy factor with different exogenously applied treatments. It was also interesting to note that the asynchronous germination lasted to 25 more days. This may be a survival adaptation of *O. arabicus* to harsh climatic conditions prevalent in a desert.

Introduction

Ochradenus arabicus has a multitude of medicinal properties, because of which it is widely used locally for curing different ailments in many countries including Saudi Arabia. However, its seed remains dormant for certain time period. To break dormancy a variety of methods are in vogue worldwide these days. Of all different methods of breaking dormancy, treatment of seed with certain chemicals including different types of plant growth regulators (PGRs) is contemplated as the most effective one. Gibberellic acid (GA_3) is the most widely used PGR to improve seed germination in different plant species (Bao & Zhang, 2011; Shen *et al*., 2012). For example, long ago apple and peach seed germination was reported to be enhanced by the application of GA_3 (Rouskas *et al.*, 1980; Mehanna *et al.*, 1985). While exploring the mechanism of action of GA_3 in improving seed germination, Arteca (1996) reported enhanced growth by GA_3 application to be mediated 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, which ultimately results in cell elongation and growth (Arteca, 1996). Despite GA_3, there are other germination enhancing chemicals available, e.g. KNO_3, thiourea, etc. However, the mode of their action and breaking dormancy is not fully understood yet. Both chemicals have been reported to overcome a few types of dormancy like seed coat or deep embryo dormant *Prunus* seed (Hartmann *et al*., 1997).

Plants found in xeric environment have developed different adaptive features for their survival therein. These plants have evolved a unique and special germination seed dispersal mechanism that is a key to their survival and development (Gutterman, 1993; Baskin & Baskin, 1998).

Limited information about *O. arabicus* seed is available regarding seed germination and seedling recruitment in nature or laboratory conditions. Therefore, the present study was conducted to understand the nature of seed and germination under laboratory conditions. This information could be useful for a large-scale propagation of *O. arabicus*.

Materials and Method

Seeds: Seeds of *Ochradenus arabicus* were collected during March-April 2011 from a derelict field, Saudi Arabia. The seed viability was estimated by Triphenyl Tetrazolium Chloride Test following the guidelines of the International Seed Testing Association (1999). The seeds (20 seed in triplicate) of *O. arabicus* were pre-soaked in distilled water for 10h and the embryos excised by removing the seed coat. They were again kept in 0.1% solution of 2, 3, 5-TTC in Petri plates (90cm dia.), wrapped with a dark paper and kept for 24h in a BOD incubator at 25°C. After 24h, the tetrazolium treated seeds were washed properly with distilled water to remove excess stain. The embryos were checked for color development under a stereomicroscope (Kyowa, Japan).

Germination studies: The seeds were treated with varying concentrations of gibberellic acid (GA_3), potassium nitrate (KNO_3), benzyl aminopurine (BAP) and thiourea. One untreated set was kept as control. The concentrations of different chemicals used were: GA_3 (25, 50, 100, 250, 350 & 500 µM), BAP (25, 50, 100, 250 & 350 µM), KNO_3 (0.1, 0.2, 0.3, 0.4, 0.5%) and thiourea (0.1, 0.2, 0.3, 0.4, 0.5%). A total of 22 treatments were used in triplicate in a completely randomized design. Similarly two more experiments were conducted at 6 month and 12 month of seed storage at room temperature. Twenty five seeds were taken for each replicate. Seed germination was recorded when germination stopped. The treated seed samples were kept on a moist filter paper in a Petri plate and incubated in a growth chamber at 25°C. Seed germination was observed up to 45 days after

sowing at a 5 day interval (Fig. 1). Emergence of radicle from seed was considered as seed germinated. All the treatments were kept in a growth chamber in the dark at 25 °C temperatures and humidity at 70-80%. Percent germination was recorded as no of seed germinated over 45 days. Analysis of variance of the data was worked out using a statistical package, SPSS and the significant differences among the treatment means were worked out the Duncan's Multiple Range Test (Crawley, 2005).

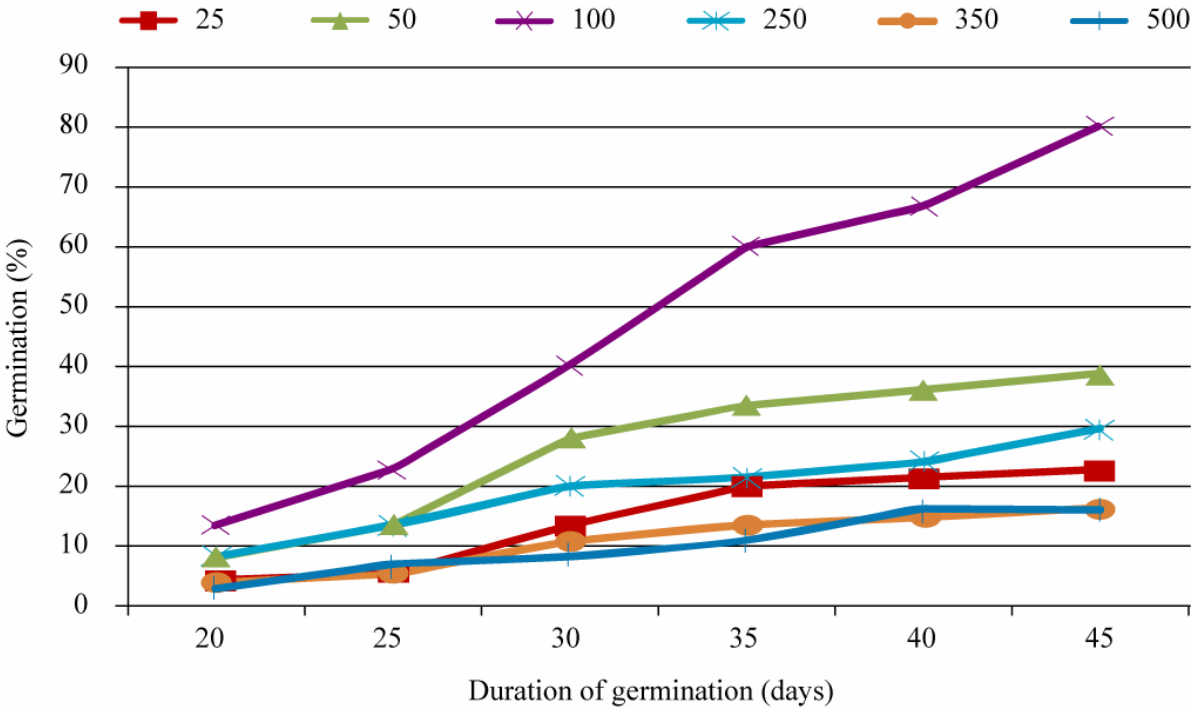

Fig. 1. Time taken for germination of seeds of *Ochradenus arabicus* treated with different levels of GA_3. (Legends show concentrations in µM).

Results and Discussion

Tetrazolium test resulted in 100% viability of the seeds stored at room temperature. Seeds sown in Petri plates started germination after 20 days of sowing in different treatments. It was found that the GA_3 was most effective in overcoming dormancy shown by the seeds of *O. arabicus*. Maximum (80-89%) germination was obtained when the seeds were treated with GA_3 at 100µM and stored up to 12 months (Table 1). As the concentration of GA_3 increased, an inhibitory effect was noted on seed germination. It was interesting to note that seeds treated with different chemicals did not germinate at the same time (asynchronous germination). The germination in most cases was delayed which is very clear from the time taken by the best treatment followed by other treatments (Fig. 1). The germination started after 20 days of sowing which continued for a further 25 days when further seed germination did not occur in any treatment. Since the treatments of BAP, thiourea and potassium nitrate were not significant, so their data were not included for time taken for germination. In control treatment, only 2.6% seed germination occurred. This shows the dormant nature of the seed of *O. arabicus* (Table 2). An increase in seed germination was observed on storage for 6 and 12 months. There was 18.6 percent germination after 12 months storage. BAP at 100µM showed 16% (0 month) germination, being the second most effective treatment after GA_3 which improved the germination 2.6% over control. Stored seed treated with BAP showed increased germination up to 38.6% (12 months). This was followed by thiourea 28.0% germination at 0.3% concentration in 12 month stored seed. In case of KNO_3, maximum germination 24.0% (12 month) was observed at 0.2% concentration. These results suggest that GA_3 is the most effective for promoting seed germination in *O. arbicus*.

In the present investigations it has been found that the seeds of *O. arabicus* require a hormone application to break dormancy. The role of GA_3 in breaking of dormancy has been documented by different workers (Sen & Mohammad, 1991; Yucel & Yilmaz, 2009; Gehan & Mona, 2011). The seeds of *O. arabicus* either do not germinate or show very low rate of germination confirming the existence of dormancy. This dormancy may be due to the presence of some inhibitors, hard seed coat, low internal hormone or underdeveloped embryos. There are reports on the application of gibberellic acid in alleviating innate and environment-induced dormancy (Khan & Ungar, 1997; Yarnia *et al*., 2012).

This study has revealed asynchronous and delayed germination by *O. arabicus* seed. In nature, this may help avoid stress and dry desert conditions. Under xeric environment, most seeds become dormant, remain in soil

and do not germinate even after rainfall. This may be a survival strategy of seeds making a seed bank under soil (Fenner, 1985). This type of seed bank was classified into transient and persistent according to the time the seed is viable in soil (Thompson & Grime, 1979). The seeds which germinate or in which viability is lost in the year of production are categorized as transient seed bank, whereas persistent seed bank are those where none, variable or few seeds germinate in the year of production. The above two types of natural seed banks can avoid the vulnerability of local extinction when vegetation on ground is removed. Therefore, they are important in restoration conservation of plants (Bakker *et al.,* 1996; Kalisz *et al.,* 1997).

Table 1. Improvement in seed germination of *O. arabicus* by GA_3 and BAP treatments.

S. No.	Concentration (µM)	Germination (%) ± SE					
		Gibberellic acid (GA_3)			Benzyl amino purine (BAP)		
		0 month storage	6 month storage	12 month storage	0 month storage	6 month storage	12 month storage
1.	25.0	22.6 ±3.5abcd	24.0±2.3abc	30.6±1.3abcd	8.0±2.3ab	13.3±1.3abc	21.3±1.3abc
2.	50.0	38.6±3.5ab	41.3±3.5ab	42.6±1.3ab	12.0±2.3b	25.3±3.5ab	33.3±4.8ab
3.	100.0	80.0±4.6a	86.6±5.8a	89.3±4.8a	16.0±2.3a	29.3±2.6a	38.6±5.8a
4.	250.0	29.3±4.8abc	34.6±5.8abc	40.0±4.6abc	6.6±1.3ab	20.0±2.3ab	22.6±1.3abc
5.	350.0	16.0±2.3abcd	18.6±2.6abcd	30.6±1.3abcd	5.3±1.3ab	16.0±2.3abc	18.6±2.6abc
6.	500.0	16.0±4.6abcd	17.3±3.5abcd	25.3±1.3abcd	NA	NA	NA
7.	control	2.6±1.3	13.3±1.3	18.6±1.3			

Mean of three replicates. Duncan's Multiple Range Test significance level at p=<0.05

Table 2. Germination of *O. arabicus* seed treated with potassium nitrate or thiourea.

S. No.	Concentration (%)	Germination (%) ± SE					
		Potassium nitrate (KNO_3)			Thiourea		
		0 month storage	6 month storage	12 month storage	0 month storage	6 month storage	12 month storage
1.	0.1	1.3 ± 1.3a	10.6 ± 2.6ab	14.6 ± 1.3abc	4.0 ± 0a	10.6 ± 1.3ab	14.6 ± 1.3ab
2.	0.2	4.0 ± 2.3a	16.0 ± 2.3a	24.0 ± 2.3a	4.0 ± 0a	13.3 ± 1.3ab	20.0 ± 2.3ab
3.	0.3	2.6 ± 2.6a	12.0 ± 2.3ab	20.0 ± 2.3ab	6.6 ± 2.6a	20.0 ± 2.3a	28.0 ± 2.3a
4.	0.4	2.6 ± 1.3a	9.3 ± 1.3ab	18.6 ± 1.3abc	5.3 ± 1.3a	14.6 ± 1.3a	20.0 ± 2.3ab
5.	0.5	1.3 ± 1.3a	8.0 ± 0.0ab	13.3 ± 1.3abc	2.6 ± 1.3a	12.0 ± 2.3ab	17.3 ± 1.3ab

Mean of three replicates. Duncan's Range Test significance level at p=<0.05

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