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SEED GERMINATION STUDIES OF *SAUSSUREA COSTUS* CLARKE, A STEP TOWARDS CONSERVATION OF A CRITICALLY ENDANGERED MEDICINAL PLANT SPECIES OF NORTH WESTERN HIMALAYA

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Abstract

Saussurea costus is critically an endangered medicinal plant species of North Western Himalaya. Its export has been prohibited as it comes under the category of red list species, according to Appendix 1 of CITES (CITES 2003). The species has been used in traditional healthcare systems of the region since times immemorial. The most widespread uses of the species include treatment of cough, cold, stomachache, toothache, ulcer and rheumatism. The roots of the species are used as an antiseptic and in controlling bronchial asthma. The Costus Oil is used in high-grade perfumes and as hair oil. It is also effective in the treatment of leprosy. In this study an attempt was made to examine the role of various treatments to overcome seed dormancy and enhance germination percentage for its effective conservation of *Saussurea costs* Clarke, a critically endangered medicinal plant species of Kashmir Himalaya. Seeds were given different physical and chemical treatments and germination was observed up to 44th day. Germination started and completed by different dates in different treatments. In treatments ch_1, ch_2, ch_3 (30,40 and 50 day chilling treatments), K_1, K_2 (Treatments treated with different concentrations of potassium nitrate, K_1= 0.2 %, K_2= 0.3%) and Control germination started and ended on different days but all these treatments took 30 days for complete germination. The best results were obtained in 50 days chilled seeds with 82 per cent germination followed by 80% and 72% germination in treatments $GA_3$1 (Gibberllic acid = 10^{-3} M) and $GA_3$2 (Gibberllic acid = 10^{-4} M), respectively. The chilling treatment ch_3 and treatment GA_3 1 were at par with each other but were statistically different from all other treatments. Treatments $GA_3$1 and $GA_3$2 with 80% and 72% germination, respectively were statistically different from each other. Treatments K_1 and K_2 were at par with each other. 24 per cent germination was observed in control treatment, but no germination was observed in seeds treated with concentrated H_2SO_4. All treatment means except treatments S_1 revealed significant differences when compared with control. Results also revealed that the rate of germination of seeds which were given different chemical treatments was enhanced in comparison to the control treatment. The coefficient of variation and mean ±standard deviation across days varied from 48.41 to 230.86 and 1.18±2.72 to 51.36±24.84, respectively.

Key words : Seed germination, Physical treatment, Critically endangered species, Chemical treatment, Conservation, Himalaya.

Introduction

Saussurea costus Clarke (Compositae) (synonymous with *Saussurea lappa*), locally known as Kuth, is a robust perennial herb of the Western Himalayas. The species is endemic to a geographically limited part of the Himalayas, and grows on moist slopes at altitudes of 2600-4000 m (amsl) (Shah, 2006; Hajra *et al.,* 1995). *S. costus* was first listed in Appendix II of CITES (the Convention on International Trade in Endangered species of Wild Fauna and Flora) on 01.07.1975 as *Saussurea lappa* and later uplisted to Appendix I in 1985. The export of *S. costus* is prohibited as it comes under the category of red list Species according to Appendix 1 of CITES (CITES, 2003). The Jammu and Kashmir has enforced a special Act, The Kuth Act, 1978 for the regulation of trade of *S. costus* (Jain, 2001). In India, *S. costus* naturally grows in the Suru Valley, Kishenganga and the upper reaches of the

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Chenab valleys in Jammu and Kashmir; with possible non-commercial sporadic occurrence in adjoining areas in Kashmir Valleys (Butola and Samant, 2010) and (Kuniyal *et al*., 2005). The species has been used in traditional healthcare systems of the region since times immemorial. *S. costus* is important medicinal plant widely used in several indigenous systems of medicine for the treatment of various ailments, *viz*. asthma, inflammatory diseases, ulcer and stomach problems. Sesquiterpene lactones have been reported as the major phytoconstituents of this species. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have convincingly demonstrated the ability of *S. costus* to exhibit anti-inflammatory (Gokhale *et al*., 2002), anti-ulcer, anticancer and hepatoprotective activities; lending support to the rationale behind several of its traditional uses. Costunolide, dehydrocostus lactone and cynaropicrin, isolated from this plant, have been identified to have potential to be developed as bioactive molecules (Ramdas *et al.,* 2012). The roots of *S. costus* have a pleasant aroma, and are used as an antiseptic and in controlling bronchial asthma, particularly of the vagotonic type. The oil extracted from the roots is known as Costus Oil, which is used in high-grade perfumes and in the preparation of hair oil. Costus Oil is also said to be effective in the treatment of leprosy. In the Himalayan states of India, the roots are used as insecticide to protect woollen fabrics and shawls, and as incense (Butola and Samant, 2010).

Keeping in view the critically endangered status and huge medicinal potential the of the species, the present investigation was undertaken to standardize some basic techniques of seed germination to overcome seed dormancy and to enhance seed germination that will finally help in effective conservation and utilization of the species.

Materials and Methods

Seeds of *Saussurea costs* were collected at maturity from different alpine natural populations *viz*, Kokernag, Pahalgam and Sonamarg of Kashmir (3000–3850 m asl) during August to September 2014. The seeds were air-dried for a fortnight at room temperature (15±2°C) and then were stored at room temperature (15±2°C). Seeds were washed with 0.1% mercuric chloride for 5-7 minutes and then with 70% alcohol for 1 minute and thoroughly rinsed with double distilled water and divided into groups of 50 seeds each. The seeds were subjected to various treatments. The surface sterilized seeds (using mercuric chloride) were soaked in distilled water for 24 hours and then subjected to chilling at low temperature (3-4ºC) for different durations {(Chilling treatment (Ch): Ch_1= 30 days, Ch_2= 40 days and Ch_3 = 50 days)} using Refrigerator (Make L.G.). Another group of seeds was treated with concentrated sulphuric acid, (S_1), for 0.5 min followed by washing in distilled water. One more group of seeds was treated with different concentrations of aqueous solution potassium nitrate (**(**KNO_3), (K_1= 0.2%, K_2= 0.3%) for 24 hours. Gibberllic acid treatment was given to one more set of seeds, which were submerged in different concentrations ($GA_3$1= 10^{-3} M, $GA_3$2= 10^{-4} M) of aqueous solution of GA_3 for 24 hours. In combined treatment**, s**eeds were treated with H_2SO_4 for 0.5 min followed by through washing with distilled water and then soaking in different concentrations of GA_3 ($GA_3$1= 10^{-3} M, $GA_3$2 = 10^{-4} M) solution for 24 hours. One treatment was kept as control (Cont). There were eleven treatments; fifty seeds in triplicate were used for each treatment. The experiment was laid in complete randomized design (CRD) with 3 replications each. After the treatment, the seeds were subjected to germination test by allowing them to germinate on the moistened filter paper. The germination of the seeds was monitored over the next forty four days at an average temperature of 15-20°C. Observation on the no. of days taken for the first seed to germinate, total no of days for complete germination and the total no of seeds germinated were noted on regular basis. The dispersion around the average germination in each treatment was measured by standard deviation. The relative effectiveness of different physio-chemical and hormonal treatments in dormancy removal and germination improvement was calculated and the seedlings were transferred to the pots.

Results and Discussion

Seed germination is a complex process, which is controlled by genetic makeup of the species and is also controlled by the conditions that prevail at the time of seed formation (Gutterman, 1995). External application of gibberellin hormone to seeds can overcome their dormancy in several species (Hassan and Fardous, 2003) and induce seed germination in some species that usually need cold stratification, light, or after-ripening (Kandari *et al*., 2012). Pre-chilling, scarification, and treatments with gibberellic acid, (GA_3) or nitric acid (KNO_3) are the standard procedures used to enhance seed germination of dormant seeds (Bhardwaj *et al*., 2016). Poor seed germination is the critical limiting factor of critically threatened medicinal plant species for large scale production and cultivation under cold conditions. Germination of seeds in *Saussurea costs* was observed up to 44th day. Different treatments took different time

periods for complete germination (table 1). In treatments ch1, ch2, ch3 (30, 40 and 50 day chilling treatments), K_1, K_2 (Treatments treated with different concentrations of potassium nitrate, K_1= 0.2 %, K_2= 0.3%) and Control germination started and ended on different days but all these treatments took 30 days for complete germination (table 1). Further, more than 50 per cent germination was observed in almost all the treatments by the 30th day of germination, hence data was analyzed for the said date. The best results were obtained in 50 day chilled seeds with 82% germination. The highest percentage may be because chilling gives stimulus and induces many genes (major or minor) to express. Various enzymes responsible for seed germination are released from genes or quantitative trait loci. These continue to express till seed is completely germinated. This is followed by successive cell division in the meristematic tissues of germinating seed. Chilling initiates an increase in the concentration of gibberllic acid (Bretzloff and Pellett, 1979). Chilling is useful to relieve primary inactiveness of many Northern hemisphere species (Baskin, 2001). It has been commonly used as a pre-sowing treatment to overcome dormancy and enhance percentage of germination of dormant seeds of many different species (Fang *et al*., 2006). The pre-chilling treatment conditions may actually be simulating the events that occur during the winter season just before the appearance of summer. Nabaei *et al*. (2011) assessed effective methods in breaking dormancy and increasing seed germination of *Rheum ribes* and found the highest germination (96%) in integrated treatment of moist chilling (25 days at 2°C and GA_3 50 mg/L). However, in the present study, chilled seeds took thirty days for complete germination. Thus, it can be presumed from the present study that pre chilling for a period of fifty days is an economic and easily applicable procedure in seed germination of the species under consideration. Further, analysis of variance revealed that all the three chilling treatments were statistically different from each other (table 2).

The next higher percentage of germination (80% and 72%) was observed in treatments ($GA_3$1 and $GA_3$2) treated with different concentrations of gibberllic acid ($GA_3$1= 10^{-3} M, $GA_3$2= 10^{-4} M), respectively. These two treatments were statistically different from each other. Treatments Ch_3 (50 day chilled seeds) and GA_3 1 were at par with each other but were statistically different from all other treatments. Plant growth regulators play a pivotal part in the mechanism/ process of germination (Ritchie and Gilroy, 1998). Gibberellic acid (GA_3) is one of the important hormones that control primary dormancy by inducing germination. Seeds when treated with gibberellins can break seed dormancy and aid in the establishment of good seedlings (Nadjafi *et al*., 2006). Fateh *et al*. (2012) also recorded better results in chilled and GA_3 treated seeds of *Echinacea purpurea* L. Gibberellins surmount seed and bud dormancy in many species, thus serving as a substitute for low temperatures, long days or red light (Salisbury and Ross, 1992). Dormant seeds which demand cold temperature treatment, dry storage following maturation as initiator or stimulator of germination are mostly treated with GA_3 to surmount their dormancy (Nadjafi *et al*., 2006). In the present study, the response to germination was influenced by concentration/proportion of applied gibberllic acid. At

Table 1 : Seed germination studies of *R S. costs* using physico-chemical treatments.

Treatments	No. of days taken for Ist seed to germinate	No. of days taken for last seed to germinate	Total no. of days taken for complete germination	Percentage of seed germination
Ch_1	8	37	30	60
Ch_2	6	36	30	71
Ch_3	5	35	30	82
S1	-	-	-	-
K1	10	39	30	58
K2	10	39	30	53
$GA_3$1	7	34	28	80
$GA_3$2	8	35	28	72
Comb 1	10	35	26	30
Comb 2	11	38	28	35
Cont.	15	44	30	24

{Chilling treatment (Ch),: Ch_1= 30 days, Ch_2= 40 days, Ch_3= 50 days: Potassium nitrate (K) with K1= 0.2 %, K_2= 0.3%: Gibbrellic acid (GA_3) with $GA_3$1= 10^{-3} M and $GA_3$2= 10^{-4} M: Combination of sulphuric acid and GA_3(Com), Com 1= H_2SO_4/$GA_3$1, Com 2= H_2SO_4/$GA_3$2: Sulphuric acid treatment,S1=0.5 min.: Control =Cont.}

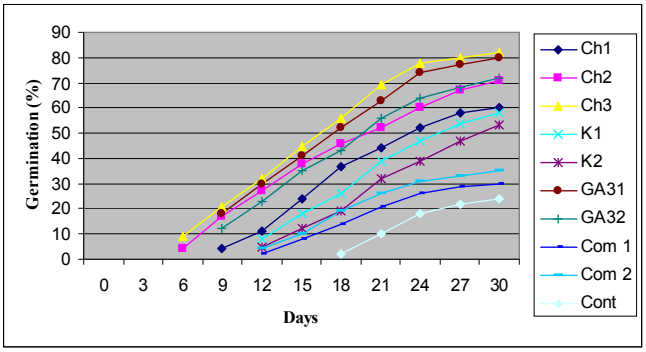

Fig. 1 : Time course of seed germination of *S. costs*.

lower concentrations of GA_3 ($GA_3$2= 10^{-4} M), germination was lower (72%) and at higher concentration ($GA_3$1= 10^{-3} M) it was higher (80%) (Table 1). Similar results were reported by Nadeem *et al.* (2000), while studying the effect of some chemical treatments on seed germination and dormancy breaking in an important medicinal plant *Podophyllum hexandrum.* Plant growth hormones are chemicals which in small quantities can regulate various plant processes in addition to seed dormancy. Different plant hormones can control different plant processes including seed dormancy and germination, growth and development of various plant parts (Agraeber *et al*., 2012). Gibberellins are mostly employed to destroy the low temperature requirements of some plant seeds and enhance their germination percentage (El-Dengawy, 2005). It plays a role in inducing enhancement of enzyme synthesis that changes stored nutrients carbohydrates which are required for quick cell respiration during germination (Bakrim *et al*., 2007). Increase in germination per centage has been also reported by other workers from studies carried out on other species, such as *Ferula gummosa* (Nadjafi *et al.,* 2006), *Sesamum indicum* (Kyauk *et al.,* 1995) and *Rumex dentatus* (Ali *et al.,* 1996). Higher per centage of germination in gibberellin treated seeds may be because GA_3 is regarded to enhance the formation of hydrolases particularly á amylase in the endosperm of cereal grains. Its breakdown is generally assumed to be an essential process of germination (Kolumbina *et al.,* 2006). Further, Finch-Savage and Leubner (2006) were of the opinion that gibberellins stimulate seed germination through amylase synthesis. Enhancement in seed germination has also been reported by other workers (Sharma *et al*., 2006 in *P. hexandrum* and Ganaie *et al*. (2011) in *Arnebia benthamii*). Similarly, Manzoor and Bhat (2013) in *Datura quercifolia*, reported that seed scarification followed by a GA_3 (200ppm) treatment as a very effective treatment wherein they observed 87% germination. Apparently, indicating that seed coat hardness is involved in imparting dormancy to seeds besides a requirement for after-ripening. During

Table 2 : Analysis of variance for seed germination in *S. costus*.

Source	d.f.	M.S.	F-Ratio	CD (5%)	C.V.
Treatments	10	2040.76	162.67	5.99	6.90
Error	22	12.54			

Table 3 : Treatment means across day 28-30 in *S. costus*.

S. no.	Treatment	Mean
1.	Ch_1	60.00
2.	Ch_2	71.00
3.	Ch_3	82.00
4.	S1	0.00
5.	K1	58.00
6.	K2	53.00
7.	$GA_3$1	80.00
8.	$GA_3$2	72.00
9.	Comb 1	30.00
10.	Comb 2	35.00
11.	Cont.	24.00

Table 4 : Mean, range and coefficient of variation of different treatments across days in *S. costus*.

Day no.	Mean± SD (Days)	Range	Coefficient of variation
0-3	0	0	0
4-6	1.18±2.72	230.86	0-9
7-9	6.54±8.21	125.64	0-21
10-12	12.90±11.98	92.87	0-32
13-15	21.00±15.75	75.04	0-45
16-18	28.54±18.60	65.19	0-56
19-21	37.45±20.96	55.99	0-69
22-24	44.45±23.18	52.15	0-78

the after-ripening of seeds, GA_3 synthesis might be stimulated to an extent necessary for dormancy removal (Sharma *et al*., 2006). The percentage germination in potassium nitrate (KNO_3, 0.1% and 0.2%) treated treatments in the present investigation was 58 and 53 per cent, respectively. However, these treatments were at par with each other. Treatment K_1 was also at par with chilling treatment chl2. Similar results were reported by Butola and Badola (2004) in *Angelica glauca* (Apiaceae), an endangered medicinal plant species wherein they reported significant increase in the rate of seed germination and promotion in mean germination time in treatments treated with KNO_3 (150 mM) and $NaHClO_3$ (30 min). Farajollahi *et al.* (2014) also reported increase in percentage germination by the application of potassium nitrate (KNO_3 0.1%) in seeds of *Calotropis persica.* Higher concentration of KNO_3 (150 mM) was significantly effective, possibly through oxidized forms

of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway (Roberts and Smith, 1977). In present study, the lowest germination (24%) was observed in the control treatment. However, no germination was observed in seeds treated with conc. H_2SO_4, indicating that acids have negative influence on seed germination. This is in contradictory to Bhardwaj *et al*. (2016), who obtained maximum percentage of germination (90%) with 5 minutes of soaking of seeds of *S. lappa* in concentrated H_2SO_4. However, similar results were observed by Nasiri and Eisavand (2001) while studying the influence of acidic treatments on germination of *Ceratonia siliqua*. Contrary to the present findings, Saied *et al*. (2008) on *Ziziphus*, Khaleghi *et al*. (2009) on Tamarind, Nasiri and Eisavand (2001) on *Albizia julibrissin* and Hojati *et al*. (2007) on *Cycas revolute* had reported sulphuric acid as the best treatment. Results also revealed that the rate of germination of seeds which received different chemical treatments was enhanced in comparison to the control treatment (fig. 1). All treatment means except treatment S_1 revealed significant differences when compared with control (table 3). The coefficient of variation and mean ±standard deviation across days varied from 48.41 to 230.86 and 1.18±2.72 to 51.36±24.84, respectively (table 4). The percentage of germination in all the treatments ranged from 0 to 82 on the day interval of 28-30 and the highest mean ±standard deviation (51.36±24.86) was also observed in day interval of 28-30.The coefficient of variation on day interval of 28-30 was 48.41 for all the treatments; indicating that the treatments are variable with respect to each other (table 4).

Conclusion

Propagation/ multiplication of a plant species increases its number of individuals and preserves its essential genetic characteristics. In this study, an attempt was made to overcome seed dormancy and to enhance germination of seeds of the critically endangered medicinal plant species (*S. costus*). From the study, it can be recommended that the most practical and useful pre-treatment for propagation of species is the chilling treatment for a period of 50 days at low temperature (3-4°C) as well as treatment of seeds with lower concentration of gibberellin. These two treatments seem to be the most practical pre-treatment methods for propagation of species on a large scale. This information shall prove beneficial for conservation of species as well as in enhancing the economy of rural people.

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