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Evaluation of germinabilities of different shrubs by some methods

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Abstract

The purpose of this paper was to determine the effect of cold stratification, GA_3 and H_2SO_4 scarification on seed germination and to assign effective method for overcoming dormancy in some shrub species. Three treatments, made were cold stratification, GA_3 and H_2SO_4 scarification treatments. Cold stratification is efficient method to enhance germination rate. Species having similar germination rate and location in same group could be assessed in breeding programs, agronomical physiological studies / applications.

Key words: Shrub, Cold stratification, GA_3 and H_2SO_4 scarification, Germination, Dormancy

1. Introduction

Shrubs, having different body structures and measures, could be easily separable from trees and other plants. Area of shrubs covers large number of species and includes cultivated and uncultivated shrub forms (Sehirali, 1989; Salisbury and Ross,1992; Seiler,G.J., 1998; Taiz and Zeiger, 2002). In scientific view, shrubs special life forms of woody plants and their height is usually less than 5-6 m. Shrubs have long been used either on banks, slopes as a barrier on traffic/garden or as forage crops for pastures in arid and semi arid regions, where shrubs arise promising plants of rangelands in. Basic principle in pasture management in arid and semi arid regions is to balance hay production and amount of grass, consumed by animals. The other principle in arid and semi arid regions where the limited amount of precipitation falls is to provide the maximum benefit from the grass and to reach its maximum production. Therefore, to establish harmony of trees, shrubs, pile rooted leguminous and shallow rooted grasses are the main purpose in rangelands (Bradbeer,1988; Gezer et al., 2005).

An important part of rangelands is threatened by desertification in Turkey. Even drastically decrease in the number of animals including sheep, and goat couldn't stop the process of degeneration. The first applicable process is to stop soil and water losses in rangelands that are under risk of erosion and desertification. In this point, one of the effective remedial applications is to allow shrub species in poor rangelands. A number of shrubs can be used as ground covers and like other ground covers, they should adapt to conditions found in the growing area. Green forage season is then determining factor for livestock production in semi arid and arid rangelands. It was revealed that forming effective insurance for short-term pasture and forage production, shrubs could plays important role to contribute quality and quantity of pasture and forage production (Salisbury and Ross,1992).

One of the restrictive factor in pasture and forage production is germinability of seeds in terms of both seed structure and environmental conditions. Biologically germination is going out of testa as a structure in embryo, capable of forming normal plant. Most of seed of plants in the face of environmental conditions such as temperature, water, oxygen to be appropriate, could germinate immediately; however, most of the seeds of plants couldn't germinate even if environmental conditions are not suitable to germinate (Bradbeer,1988; Lee et al., 2006). Eisvand et al. (2006) pointed out that seed, just reached maturity usually needs a certain period to germinate. Besides, Govind Naik et al. (2010) stated that when successful seed propagation is made, hard seed coat is main obstacle in good enough germination.

Despite presence of embryo and endosperm of seeds to germinate, internal or external factors of seed causes plant growth stagnation is called dormancy. In other words, induced or enforced dormancy mostly occur and it is

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natural phenomena in pasture and forage plant seeds that most of the show dormancy in various degree and type. Seed germination is initial development in plant life of plants including shrubs and an important part of plants have dormant seed (Keller and Kollman, 1999; Keshtkar et al.,2008). Impermeability of testa for water and oxygen, mechanically prevention of embryo by testa, incomplete growth in embryo, inhibitors in seeds for germination and growth are almost seen as inhibitors in germination (Salisbury and Ross,1992; Prased et al.,1996). Once seeds having such germination problems (dormancy, viability), some treatments such as H_2SO_4 scarification (Song et al., 1990; Güneş et al.,2009) cold stratification (Rouhi et al., 2010), GA_3 (Prased et al.,1996; Kabar, 1997) generally allow to overcome dormancy. Song et al. pointed out that (1990) using H_2SO_4 scarification shows little effect to promote germination or to break down dormancy in many species. Pacheco and Metos (2009) found that H_2SO_4 scarification is efficient treatment to overcome dormancy in *Apeiba tibourbov* seeds. But this treatment isn't expected to break dormancy in all plant seeds. It is inefficient in most plant species. Having hard-structure seed coat, some plant species doesn't germinate, and their hard cuticle doesn't allow germinate of water. So cold stratification in wet send is safe method to break dormancy (Bradbeer,1988; Lee et al.,2006). Keshtkar et al. (2008) reported that significant decreases were recorded in Ferula ovina and Ferula gummosa in both cold stratification and GA_3 treatments. Besides, cold stratification significantly increased germination percent and this percent increased with increasing stratification period (Keller and Kollman, 1999; Eisvand et al.,2006). Studies related to GA_3 treatment cited that GA_3 treatment increases germination rate of many plants (Chvancern et al.,2004; Prased et al.,1996; Salisbury and Ross,1992) and significantly affects physiological and metabolic characteristics of seeds(Seiler,1998, Chvancern et al.,2004). Rouhi et al(2010) found that cold stratification for 49 days is better treatment on breaking dormancy of waterlily seeds than GA_3. Zincirkıran et al,(2010), concluded that eight weeks of moist stratification gave best results after 30 minutes H_2SO_4 scarification to remove physical and physiological dormancy in seeds of C.siliwuastrum L. Gezer et al.(2005) revealed that cold stratification for at least 3 months is necessary to obtain enough germination in *Sorbus domestica* L. Zapata el al. (2008) found that scarification with H_2SO_4 scarification and cold stratification had no effect on *Euphorbia lathyris* L., whereas hydration and germination in constant darkness at 23 °C gave best germination rate. The purpose of this paper was to determine the effect of cold stratification, GA_3 and H_2SO_4 scarification on seed germination and to assign effective method for overcoming dormancy in some shrub species.

2. Materials and methods

This study was carried out in laboratory and greenhouse conditions of Anatolian Agricultural Research Institute in Eskişehir province of Turkey in 2009. Seeds of 30 shrub species, were collected in Eskişehir, Bilecik and Afyon provinces during the years of 2008-2009. Shrub species gathered were given in Table 1.

Table 1. Shrub species gathered and used in the study

No	Shrub Species	No	Shrub Species
1	*Cistus creticus L.*	**16**	*Mahonia aquifolium* (Prush.) Nutt.
2	*Phillyrea latifolia L.*	**17**	*Rubus caesius* L.
3	*Buxus sempervirens L.*	**18**	*Jasminum fruticans* L.
4	*Smilax excelsa L.*	**19**	*Crataegus marginatus* L.
5	*Sorbus domestica L.*	**20**	*Rhus coriaria* L
6	*Paliurus spina-cristi* Miller.	**21**	*Crataegus marginatus* L.
7	*Gypsophila sphaerocephala* Fenzl ex Tchihat.	**22**	*Rosa canina* L.
8	*Cotoneaster integerrimus Medik.*	**23**	*Euonymus europaeus* L.
9	*Clematis orientalis L.*	**24**	*Pyracantha coccinea* Roemer.
10	*Gonocytisus angulatus L.*	**25**	*Viscum album L.*
11	*Colutea cilicica* Boiss.	**26**	*Sorbus aria L.*
12	*Dorycnium graecum* L.	**27**	*Acer campestre L.*
13	*Cephalaria procera* Fısch.	**28**	*Euonymus latifolius* L.
14	*Globularia trichosantha* Fisch.	**29**	*Cotoneaster horizontalis Dcne.*
15	*Colutea cilicia* Boiss.	**30**	*Cotaniester lacteus* W.W. Sm.

Seeds of shrub species were sterilized by soaking in 5% sodium hypochlorite (NaOCl), solution for 10 min then rinsed with sterilized water before treatments. Seed germination processes after all treatments, Though GA_3 and H_2SO_4 scarification were conducted in laboratory and cold stratification treatment was performed in greenhouse conditions. Three treatments, made were cold stratification, GA_3 and H_2SO_4 scarification. **Cold stratification:** Seeds were kept at 3-4°C in wet sand conditions for 60 days before the germination test (Seiler,1998, Chvancern et al.,2004; Keshtkar et al., 2008; Subaşı and Güvensen 2010). **GA_3 treatment:** Seeds were treated with GA_3 (SIGMA, Germany, 90%) was added to distil water (500ppm). Seeds were soaked in GA_3 solution in light at room temperature for three days (Kabar, 1997; Köse, 1998; Eisvand et al.,2006). **H_2SO_4 scarification:** Seeds were immersed in H_2SO_4 scarification (98%) for 10 min, then rinsed with distilled water (Song et al., 1990; Güneş et al.,2009). When all treatments were

finished, seeds were put into germinator with alternative light/darkness and temperatures of 10°C N, 22°C D. Germinated seeds were counted and removed every 24 h for 60 days. For germination applications, 100 seeds were used for germination tests having three replication. Seeds in sterile petri dishes were put in double layered Whatman No.1 filter paper and moistened with 5 ml of distilled water. Once tip of the radicle was grown free of the seed coat, seed was accounted for germinated (Auld et al., 1988). Mean germination time was calculated as follows (Keshtkar et al.,2008):

Gr: Σ (number germinating since n-1)/ n **Where:**

Gr: Germination rate, **n:** The days of incubation

3. Results

Three applications to increase germination on some seeds of shrub were made and variance analysis table was given in Table 2.

Table 2. Variance Analysis Table of Species and Treatments

Source of Variation	Degree of Freedom	Means of S.S.	F Value
. **Replication**	1	49.09	71.26*
Application	2	5316.24	7717.19**
Error-1	3	0.69	
Species	29	990.17	385.38**
Appl x Species	58	525.79	204.64**
Error	87	2.57	
Mean	179	391.72	
CV(%): 116.19			

As seen in Table 2, the effect of treatments applications on germination rates, differences between shrub species and their interaction were found to be important at 1 %. Bigger F value was taken from shrub species. It assign that there are big variations in genotypic structures Keller and Kollman, (1999) says that different shrub species naturally have various genotypic structures causing different germination rate and growth shrub genotypes are mostly wild plants and in uncultured characteristics having seed coat or dormant embryo, some shrub species couldn'germinate until dormancy, originated from either hard-thick structure of seed coat or embryo is broken down; germinability could increase when suitable environmental conditions occur or seeds need some applications such as cold. GA_3 or H_2SO_4 scarification etc. Cold stratification is safe and important method to break down and to increase germination rate (Keshtkar et al.,2008). Besides, the effect of treatments on shrub species for germination rates was given in Table 3.

Treatments significantly caused on germination rates of species ($p<0.01$) and the highest effect on germination rate belonged to cold stratification (21.74%), whereas H_2SO_4 scarification had the lowest one (2.98 %). Differences among species for germination were found to be significant at 1%. Due to differences in genotypic characteristics in shrub species, plant behaviors such as germination and growth are naturally different (Keller and Kollman, 1999; Eisvand et al.,2006). Significant difference among species ($P<0.01$) corroborate this expectations or idea. The highest germination rate was taken from *Clematis orientalis* L. (46.47%). However *Buxus sempervirens* L. (1.80%), *Crateagus marginatus* L. (0.00%), Rhus cariaria L. (0.40%), *Crateagus marginatus* L. (0.40%), *Rosa canina* L. (1.00%), *Euonymus europaeus* L. (0.40%),*Viscum album* L. (0.40%), *Acer campestral* L. (0.40%), *Euonymus latifolius* L. (0.80%), and *Cotaniester lacteus* W.W. Sm.(0.40%) showed no germination rate. Having no germination rates species may be dormant or they don't have viable seed. Once viability tests such as tetrazolium are seed, the circumstances of seed having no germination rate could be determined.

Interaction between shrub species and applications was determined as significant ($P<0.01$). Germination rates for each applications seemed to be significantly different for instance, germination rate in *Clematis orientalis* L. was 46,47%. But in cold stratification no germination occurred in *Acer campestre* L. (0.40%) in all applications. Though similar germination rates of ranges in species for each application were expected, these contradictory results made interaction significant at 1%. It's hard to expect that all species have some or similar genotypic characteristics (Govind Naik et al., 2010) and most species eventually have different germinability (Salisbury and Ross,1992).

Similarities and dissimilarities of species with regard to germination rates were given in Figure 1. This figure showed that species were divided into four groups in terms of germination rates. *Clematis orientalis* L, *Phillyrea latifolia L, Pyracantha coccinea* Roemer, *Gonocytisus angulatus* L, *Sorbus domestica L.* joined in Group 1. Besides, *Smilax excelsa L, Globularia trichosantha* Fisch. , *Cotoneaster horizontalis* Dcne., *Gypsophila sphaerocephala* Fenzl ex Tchihat, *Colutea cilicia* Boiss., *Cistus creticus L, Jasminum fruticans* L. and *Sorbus aria* L. denoted similar results and get into some group (Group 2). Another group consisted of *Paliurus spina-cristi* Miller, *Cephalaria procera* Fisch et Lall, had similar germination rates for three applications and ranged in Group 3.

Table 3. The Effect of Treatments on Shrub Species for Germination Rates

	Species	Cold	GA_3	H_2SO_4	Mean	
1	*Cistus creticus* L.	63.40	15.00	0.00	26,13	E
2	*Phillyrea latifolia* L.	30.10	70.00	14.60	38,23	B
3	*Buxus sempervirens* L.	1.20	3.00	1.20	1,80	MO
4	*Smilax excelsa* L.	34.60	8.00	14.60	19,07	GH
5	*Sorbus domestica* L.	81.20	3.00	0.00	28.07	D
6	*Paliurus spina-cristi* Miller.	0.00	19.00	5.70	8.23	K
7	*sophila sphaerocephala* Fenzl ex Tchihat.	41.20	16.00	1.20	19,47	GH
8	*Cotoneaster integerrimus* Medik.	1.20	0.00	7.90	3,03	LM
9	*Clematis orientalis* L.	79.00	57.00	3.40	46,47	A
10	*Gonocytisus angulatus* L.	39.00	26.00	25.70	30,23	C
11	*Colutea cilicica* Boiss.	19.00	8.00	3.40	10,13	J
12	*Dorycnium graecum* L.	5.70	0.00	1.20	2,30	LN
13	*Cephalaria procera* Fisch.	0.00	21.00	0.00	7,00	K
14	*Globularia trichosantha* Fisch.	34.60	0.00	1.20	11,93	IJ
15	*Colutea cilicia* Boiss.	41.20	25.00	3.40	22,53	F
16	*Mahonia aquifolium* (Prush.) Nutt.	0.00	3.00	0.00	4,00	L
17	*Rubus caesius* L.	0.00	3.00	0.00	1,00	NO
18	*Jasminum fruticans* L.	54.60	10.00	1.20	21,93	F
19	*Crataegus marginatus* L.	0.00	0.00	0.00	0,00	O
20	*Rhus coriaria* L.	1.20	0.00	0.00	0,40	NO
21	*Crataegus marginatus* L.	1.20	0.00	0.00	0,40	O
22	*Rosa canina* L.	0.00	3.00	0.00	1,00	O
23	*Euonymus europaeus* L.	0.00	0.00	1.20	0,40	O
24	*Pyracantha coccinea* Roemer	12.30	41.00	0.00	17,77	H
25	*Viscum album* L.	0.00	0.00	1.20	0,40	O
26	*Sorbus aria* L.	59.00	1.00	0.00	20,00	G
27	*Acer campestre* L.	1.20	0.00	0.00	0,40	O
28	*Euonymus latifolius* L.	1.20	0.00	1.20	0,80	NO
29	*Cotoneaster horizontalis* Dcne.	41.20	0.00	0.00	17,73	I
30	*Cotaniester lacteus* W.W. Sm.	0.00	0.00	1.20	0,40	O
Mean		21.74	11.00	2.98		11.90
L.S.D. (%): Treatments: 0.75, Species: 2.45, Treatments x Species: 3.46						

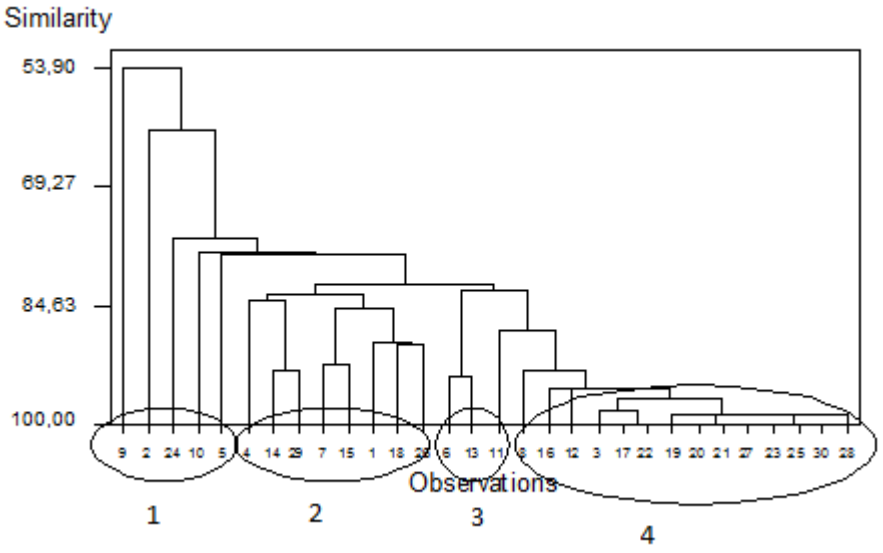

Figure 1. Dendogramic analysis of shrub species

Moreover, *Cotoneaster integerrimus* Medik., *Mahonia aquifolium* (Prush.) Nutt**,** *Dorycnium graecum* (L)Se, *Buxus sempervirens L, Rubus caesius* L, *Rosa canina* L, *Crataegus marginatus* L, *Rhus coriaria* L, *Crataegus marginatus* L, *Acer campestre* L, *Euonymus europaeus* L, *Viscum album L, Cotaniester lacteus* W.W. Sm. and *Euonymus latifolius (* L.) Mill Engl. were evaluated in Group 4.

Determining similarities of applications by dendogram were showed in Figure 2. Dendogramic analysis revealed that GA_3 and H_2SO_4 scarification applications were considered in Group 2, rather than cold stratification in Group 1.

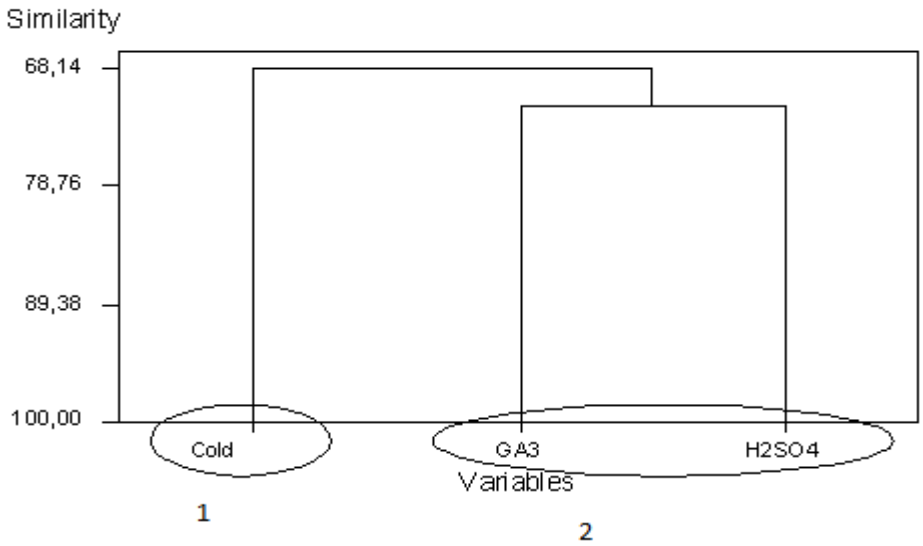

Figure 2. Dendogramic analysis of treatments.

This mean that cold stratification gave different germination results than that of GA_3 and H_2 SO_4. All analysis assigned that cold stratification alone is more efficient in germination of shrub species.

4. Conclusions

As a result, cold stratification treatment was found as the most efficient method to increase germination rate in shrubs and it could be safely used in when germination problems are come across in dormant seeds. Besides, GA_3 and H_2SO_4 treatments are also be used in similar germination studies. Shrub species in same same groups could be assessed that they have similar germination characteristics. Further studies are needed to determine genotypic and phenotypic characteristics of shrub species..

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