Techniques to overcome hard seed coat dormancy and improving seed germination in Liquorice (*Glycyrrhiza glabra L.*)

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Abstract - *Glycyrrhiza glabra* is an important medicinal plant and used as a constituent of ayurvedic and unani medicine .Indigenous cultivation of liquorice (*Glycyrrhiza glabra*) is very low because of poor seed set and germination. The seed showed dormancy and causes a big challenge in seed germination.Commercial plantation is done by stolon.The seeds showed exogenous dormancy.The water imbibitions rate of nuts was moderate (0.410-0.510g/g seeds/day) which was slightly improved by acid scarification (0.483-0531g/g seeds/day) hot water treatment (0.443-0.553 g/g seeds /day).Thus the woody wall of nut is permeable to water and the water is available to the seeds the nut. The seeds taken under study were subjected to quick viability test (TZ) which showed 93-96% seed viability and the low value of electrical conductivity of the seeds leachates (0.217-0.185µmho/cm^1) also indicated the high intactness and viability of the seeds.The chipped seeds treated with GA_3 in all three concentrations 100, 300 and 500 ppm securing significantly higher germination percentage as compared to cheeped seeds alone indicating synergistic effect. No significant differences in germination percentage were observed between chipped seeds treated with chemical KNO_3 & Thiourea and the chipped seeds alone except KNO_3 (0.5%) treatments.

Key Words: Dormancy, Germination , Acid scarification , Vigour index

I. INTRODUCTION

Liquorice (*Glycyrrhiza glabra*) is the commercially important perennial plant grassy or semi bushy species belonging to family leguminoceae, sub-family Papilionaceae commonly known as mulethi,yashtimadhu. The genus name is driven from the Greek words, glycy or glulus meaning sweet and rhiza meaning root (Crusheva and Parvanov1978). In India it is widely cultivated from sub Himalayan tracts from chenab Westward to Myanmar and Andaman Islands.Its roots, runners and rhizomes are commercially important parts that provides Glycyrrhizin which is widely used in pharmaceutical Industries these components are used in medicine for its non-nutritive sweetness and anti-allergic and anti-inflammatory effects as treatment of bronchial asthma, allergic, dermatitis and eczema.These are also used as food in confectionery industry such as sweets, alcohol-free drinks and in the tobacco industry (Rastitel'nye, 1987).The objective of this research was to evaluate the effect of seed scarification and constant temperature regimes on licorice seed germination. Liquorice is reproduce by rhizome and seeds but in a natural stand the seed germination is very low due to dormancy caused by hard seed coat which require scarification before sowing (Gupta *et al*.1997) (Boe and wynia 1985). Experiments have been conducted to break liquorice seed dormancy by chemical and mechanical scarification treatments.

II. MATERIALS AND METHODS

The mature pods of Glycyrrhiza glabra were collected from NBPGR , Pusa Campus , New Delhi and other reliable sources for this study . The seeds showed high level viability percentage (93-96%) as tested with Triphenyl tetrazolium chloride (TTC).The seeds were subjected to the following treatments:

Mechanical Scarification:-In mechanical scarification the surface of the seed were scarified by chipping or rubbing on the rough surface or sand paper.

Hot water treatment:-The seeds were soaked in hot water (70^0C) for 10 ,30 and 60 minutes duration.

Acid scarification- Seeds were soaked in three concentration (25%,50% and100%) of sulphuric acid for 5 min. duration.

Chipping + Chemical treatment:-The seeds were chipped and then treated with different concentrations of GA_3 , KNO_3 and Thiourea for 24 hr. soaking duration.

I. **Gibberelic acid:-**seeds were soaked in 100 , 300 and 500 ppm GA_3 concentration for 24 hr and then plated them on the moist filter / tissue paper for germination.

II. **KNO_3:-** seeds were soaked in 0.1%, 0.5% and 1.0% concentration of KNO_3 for 24 hr. and then plated them on the moist filter / tissue paper for germination.

III. **Thiourea :-** seeds were soaked in 0.1% ,0.5% and 1.0% thiourea concentration for 24 hr. and then plated them on the moist filter / tissue paper for germination.

Seed germination test :-Germination test was conducted with four replicates of 25 seeds each , following the ISTA method at 27^0 C (Anonymous 1985).The germinated seeds were categorized in to normal ,abnormal , dead and hard seeds

counted every day .Germination percentage was recorded on the basis of normal seedling only .Seeds were considered to have germinated when the redicle has emerged out.

Vigour measurement :-Vigour index was calculated as the product of seedling vigour (root + shoot length) and germination percentage.

Vigour index = Seedling Vigour (root + shoot length) X germination percentage

Speed of germination:- For calculating the speed of germination the germination counts were taken every 24 hr. and seeds were considered germinated when 1 mm radical emerge .An index was computed for each treated lot by dividing the number seedlings removed each day by the day after planting on which they were removed.

$$\text{Speed of germination} = \frac{\text{No.of seedling removed daily}}{\text{Days after planting}}$$

Statistical analysis:-The data recorded for different parameters were analysed statically by method of analysis of variance as described by Cochran and Cox (1957) under completely randomized block design.

III. RESULTS AND DISCUSSION

The seeds of *Glycyrrhiza glabra* under study showed 100 percent viability as tested with triphenyl tetrazolium chloride (TTC)**.** The seeds of *Glycyrrhiza glabra* medicinal plants studied under two lots showed very low or nil germination under favourable germination conditions. The data pertaining to the effect of different treatments to overcome the dormancy and improving the germination are presented in table1. The seeds were scarified to overcome the seed coat dormancy constraints. Mechanical scarified nuts showed (38-40%) germination, seedling vigour (2.5-2.7) and seedling vigour (100-102).Veena *et al*.(1997) also reported a little effect on germination (36.6 %) of mechanically scarified seeds.

Hot water treatment 60 min. showed 25-30 % germination, seedling vigour (2.3-2.6 cm) and vigour index (65.0-69.0) and speed of germination (6.0-8.3) similar finding were recorded in *U.picta* seeds treated with hot water for 10 min. resulted in 40% germination. These results indicate that hot water treatments were slightly effective in promoting germination in *G.glabra*.(Ahire *et al*.2009).Boiling water treatment for two minutes was reported to be most effective treatment on germination percentage of Licorice (81.33%) increase in germination percentage compared to control.(Rafieiolhossaini Mohammad *et al*.2015).Results of this scarification studies indicated that seed coat was the major barrier to liquorice seed germination seed coat hardness of this type is also common among perennial plant including common milkweed (*Asclepias syriaca L*.) field bind weed (*Convolvulus arvensis L*.).The crop however a predominantly propagated through vegetative parts mostly rhizomes, stolons or other cuttings. This propagation methods is disadvantages due to the requirements of economically valuable part of the plant, slow growth of rhizomes and their reduced reproductivity due to unfavorable climate and soil conditions (Gupta *et al.*,1997;Duke,1981). Interestingly a study conducted by Gladysev (1991) showed that plants generated from seed were comparable to those produced by root, however seed dormancy makes it hard to grow various methods of breaking seed dormancy were described including mechanical scarification through use of abrasive materials rushing of seed coat by special rolling presses sulphuric acid (H_2SO_4) treatments, hot-water etc.(Shamsutdinov1996;Gupta *et al.*1997). *G.glabra L.* seeds soaked in sulfuric acid for 10 to 60 minutes germinated at 19-20^{0}C (shunkurullaev and khamdamov,1976).Maximum germination (98.3%) was obtained with seeds treated with 40 minutes. Only 7% of control treatment seeds germinated.Khudahibergenov and Mikhahilova(1972) showed that untreated seeds of *G.uralensis L*. have (11%) germination in the laboratory and (9%) in the field.Treatment in concentrated sulfuric acid (H_2SO_4) increased germination from (60 to 94%).

The maximum germination percentage (75-80%),vigour index (192.0-208.0) and speed of germination (16.7-17.3) were observed in the seeds scarified with100% H_2SO_4 for 5 minutes treatment. The most appropriate combination was pre-treated seeds with 100% sulphuric acid for 5 minutes break the seed coat without damaging the seed viability (Veena *et al*,1997). Verma *et al.* (2001) reported that acid treatment was effective in breaking *Glycyrrhiza glabra* dormancy and obtained (88%) germination. Chemical scarification increased percent germination significantly to (90-95%) 45min soaking (Ghadiri H.and Bagherani N.,2000). Artificial softening of the seed coat is being extensively used to reduce hard seededness in seed lots and to improve the germination rate (Rolston 1978;Tomar and Singh1993; Copeland and Mc Donald 1995; Jin *et al*., 2006). This kind of dormancy can be erased by various physical and chemical dormancy breaking treatments (Das and Saha 1999).Among such treatments it has already been reported that mechanical scarification and concentrated sulfuric acid treatments are the most common and convenient scarification methods for breaking *Glycyrrhiza* species seed dormancy as testa-imposed such as *G. glabra* (Gupta *et al.*1997;Ghadiri and Torshiz 2000;Verma *et al.*,2001) and *G. uralensis* (Luo *et al*.2000;Jin *et al*.2006) .

The mechanically scarified seeds following 24hr soaking in 0.5% KNO_3 solution exhibited the germination percentage (42-49%) against mechanical scarification alone (38-40%). KNO_3 solution treatment was ineffective in reducing the percentage of hard seeds in *G.uralensis* (MAO Pei-sheng *et*

*al.*2008) but chipped seed treated with KNO_3 solution enhanced germination percentage as compared to cheeping seeds alone. KNO_3 was also least effective in breaking the hard seededness in *G.glabra* (Gupta *et al.*1997) .Similar effects on roots and shoot vigour has been observed in Licorice but no effect on germination percentage.(Gupta *et al.*1997)

The mechanically scarified seeds following 24hr soaking in GA_3 (300ppm) solution exhibited the germination percentage (62-65%) against mechanical scarification alone (38-40%).But the application of gibberellic acid and potassium nitrate both along with scarification (flooding) pretreatment had no effect on Licorice dormancy (Rafieiolhossaini Mohammad *et al.*2015).

The application of exogenous GA_3 treatments was ineffective in promoting germination percentage in *U.picta* seeds which indicated the higher level of endogenous GA_3 germination indicating no need of GA_3 application for this purpose.(Ahire *et al.*2009)

Table:1- Effect of different pre showing seed treatments on germination , root / shoot length (seedling vigour), vigour index and speed of germination in different seeds lot of Liquorice (*Glycyrrhiza glabra L.*)

Treatments	**Germination (%)**		**Shoot length (cm)**		**Root length (cm)**		**Total**		**Vigour index**		**Speed of germination**	
Seeds lot	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	0	0	0	0	0	0	0	0	0	0	0	0
Scarification (Mechanical)	40	38	1.2	1.5	1.3	1.2	2.5	2.7	100.0	102.6	9.0	9.5
Hot water												
10 min	0	0	0	0	0	0	0	0	0	0	0	0
30 min	0	0	0	0	0	0	0	0	0	0	0	0
60 min	30	25	1.1	1.4	1.2	1.2	2.3	2.6	69.0	65.0	8.30	6.0
Acid Treatment (5minutes)												
H_2SO_4 25%	15	10	1.1	1.5	1.5	1.1	2.6	2.6	39.0	26.0	2.6	2.1
H_2SO_4 50%	20	25	1.5	1.3	1.1	1.3	2.6	2.6	52.0	65.0	4.5	5.7
H_2SO_4 100%	80	75	1.5	1.2	1.1	1.2	2.6	2.4	208.0	192.0	16.7	17.3
Chipping + Chemical												
GA_3 100 ppm	45	45	2.8	1.7	1.7	1.8	4.5	3.5	202.2	157.5	8.9	11.3
300 ppm	65	62	2.9	2.6	1.8	1.1	4.7	3.7	305.5	229.4	13.0	13.2
500 ppm	60	55	2.6	2.6	1.5	1.2	4.1	3.8	246.0	209.0	11.9	12.6
KNO_3 0.1%	40	32	2.0	2.0	1.4	1.0	3.4	3.0	136.0	96.0	8.5	7.8
0.5%	49	42	1.8	2.5	1.2	1.1	3.0	3.6	147.0	151.2	8.0	9.4
1.0%	45	35	2.4	2.8	1.0	1.0	3.4	3.8	153.0	133.0	7.6	10.0
Thiourea 0.1%	39	35	1.0	1.8	1.8	1.0	2.8	2.8	109.2	98.0	7.0	8.2

0.5%	45	39	1.4	1.6	0.9	0.9	2.3	2.5	103.5	97.5	7.8	8.0
1.0%	35	25	1.4	1.9	1.0	1.0	2.4	2.9	84.0	72.5	7.3	7.5
CD at 5%	3.06	2.29	0.13	0.11	0.065	0.06	0.22	0.21	7.23	9.33	0.52	0.47

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