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Effect of physical and chemical scarification and ageing on hardseededness in *Clitoria ternatea*

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Abstract
Clitoria ternatea is an important range legume for nutritional improvement of rangelands under semi-arid regions. It is perennial and hardy in nature which can survive well under semi-arid conditions on low fertile, sandy soil with limited soil moisture. The dry matter production potential is ranging from 2-3 t/ha with high protein content of 18-20% and digestibility of about 80 per cent. Due to presence of hard seed the field emergence of fresh seed of *C. ternatea* is less and/or delayed, resulting in poor field establishment. Fresh seed (<3 month age) of *C. ternatea* was exposed for different pre-treatments: physical and chemical scarifications *viz.* nicking (making cut on seed coat), rubbing between sand paper, soaking in H_2SO_4 for 5-20 min., soaking in hot water (80°C) for 5-20 min. and in boiling water for 5 min. for evaluation of their effect on removal of hardseededness. For study of effect of ageing on level of hardseededness fresh seed, one, two, three and four year old seed were evaluated for germination. The findings revealed that highest germination (90%) was achieved by nicking followed by sand paper scarification which increased germination up to 70.5% against control (6.5%). Scarification with conc. H_2SO_4 for 10 min. was at par with sand paper scarification but proportion of abnormal seedlings was increased by 3.3%. Highest normal seedlings (70.5%) were observed in two year old seed against fresh seed (6.5%). The proportion of hard seed decreased up to 8% in four years old seed but on the other hand normal seedlings also reduced up to 56.3 per cent.

Keywords: Germination, Hardseededness, Pre-treatment, Range legume, Seed coat

Introduction

Clitoria ternatea also known as *titaly matar*, butterfly-pea and *Aparajita* belongs to family *Leguminosae. It* is a vigorous herb with fine twining stems and strong persistence. It was originally treated as a cover crop. Due to presence of hard seed, it can survive well under rainfed conditions on low fertile and sandy soils. *Clitoria* is not only a promising range legume for nutritional improvement of rangelands under semi-arid regions, but it is also used for short and medium-term pastures, as green manure and protein bank. It can also be used for cut-and-carry, conserved as hay and dry leaf meal. The dry matter production potential is ranging from 2-3 t/ha/year with about 18-20% protein content and 80% digestibility.

Mature seeds of many plant species, particularly legumes, do not germinate readily under favourable environmental conditions, because they are impermeable to water and/ or gases or possess hard seed-coat that mechanically constraints the embryo. This attribute has been called hard seed coat dormancy, hardcoatedness, hardseededness, dormancy imposed by the seed-coat, seed-coat impermeability and exogenous dormancy (Argel and Paton, 1999). Due to presence of hardseededness in *C. ternatea* its field emergence is less and germinates unevenly over a long period when fresh seed is used which results in poor field establishment. Seedlings that germinate early may be well established while the late germinating seedling may be vulnerable to the soil moisture stress during receding of monsoon and starting of the winter season. Dormancy is also a major challenge in seed testing and gene bank operations as dormant seed will not germinate even under optimum conditions (Argel and Paton, 1999). Seeds of many wild species of legumes are most likely to have hard seed, and pretreatments for dormancy breaking are required before testing and sowing (ISTA, 2003). Much research has been done on breaking dormancy of hard seeds of temperate legumes but work on tropical and subtropical forage species is limited. Therefore, present study was undertaken to know the effect of different physical and chemical pre-treatments and ageing on hardseededness in *C. ternatea*.

Materials and Methods

Seed samples of *C. ternatea* were procured from Indian Grassland and Fodder Research Institute (IGFRI), Jhansi during 2008. The fresh seed of both the genotypes were tested for seed germination at Western Regional Research Station of IGFRI, Avikanagar under laboratory conditions as per ISTA (2004). The fresh seed was exposed to various physical and chemical pre-treatments to study their effectiveness in removal of hardseededness of *C. ternatea*.

Physical and chemical pre-treatments: *Nicking:* A cut was made on seed coat at opposite of the micropyle manually with the knife. *Sand paper scarification:* The seed was rubbed between sand papers manually until the shinny and smooth seed surface become rough and dull. *Soaking in hot water*: Seed was soaked in hot water at 80ºC for 5, 10, 15 and 20 minutes and gently stirred during the soaking. *Soaking in boiling water*: Seeds were soaked in boiling water (100˚C) for 5 minutes. *Soaking in concentrate H_2SO_4*: Seeds were submerged in concentrate H_2SO_4 for 5, 10, 15 and 20 minutes. The seed was rinsed for 3-4 times with tap water after completion of treatment.

Ageing effect on germination and hardseededness: Various lots of fresh seed of *C. ternatea* were stored in cloth bags at ambient storage condition to study the effect of age (storage) on germination and level of hardseededness. The seed germination and vigour parameters were studied in differentially aged seed of 0, 1, 2, 3 and 4 years under ambient conditions. The experiments were conducted in completely randomized design with four replications. For germination test 100 seeds were kept between papers (BP) in each replication at 25ºC for 10-days under dark condition. To estimate vigour, seedling length and dry weight were observed in 10 randomly selected normal seedlings from each replication as per ISTA (2004). The data were subjected for analysis of variance as per standard procedures.

Results and Discussion

Effect of physical and chemical pre-treatments: Among the different methods applied for breaking hardseededness, highest germination (90%) was achieved by nicking against control (6.5%). The increased seed germination by physically scarified seeds through nicking and sand paper suggested that seed dormancy in *C. ternatea* is mainly due to presence of hard seed coat which renders the seed testa impermeable to water and gases required for initiation of germination process. Nagaveni and Srimathi (1980) reported that pre-treatment of hard seed causes degradation of the seed coat thus allowing greater permeability to water. In the present study, scarification of seed between sand papers increased normal seedlings up to 70.5 per cent. Similar results were reported by Wang *et al.* (2011) in five wild *Vigna* species. Ghassali e*t al.* (2012) studied effectiveness of boiling water, conc. H_2SO_4 and mechanical scarification for removing hardseededness in 14 *Acacia* species where highest germination (95%) was achieved by sulphuric acid treatment. Deminicis *et al*. (2006) observed that hand scarification with sand paper was the best method for overcoming dormancy in *C. ternatea* and seven other tropical forage legumes. Omran (2013) recorded 70% germination in *Leucaena leucocephala* with the application of mechanical scarification compared to 40% in control.

Present study revealed that seed soaking in concentrate H_2SO_4 for 10 minutes increased normal seedling up to 71.3% with low amount of abnormal seedlings (Table 1). The duration of pre-treatment with sulfuric acid vary from a few seconds to several hours, and in majority of cases pre-treatment periods are between 1 and 20 minutes (Ellis *et al.,* 1985). Concentrate sulfuric acid scarification for breaking seed dormancy for *Stylosanthes guianensis* was recommended by the ISTA (2004). Botsheleng e*t al.* (2014) observed that mechanical scarification increased germination of *Afzelia quanzensis* up to 100% while soaking seed in H_2SO_4 for 6 min also increased germination significantly over control. Lorato *et al.* (2014) studied the effectiveness of pre-treatments (cold water, hot water and concentrate sulphuric acid) on the germination of three *Acacia* species. Concentrate sulphuric acid increased the germination of *A. erioloba* up to 87% and *A. nigrescens* up to 30% while, hot water for 9 min enhanced germination by 30% in *A. tortilis.* Pietrosemoli and Mendiri (1997) observed that treatment with sulfuric acid for 5 min improves germination of *C. ternatea* seeds. Likoswe *et al.* (2008) reported that nicking and soaking in cold water for 12 h gave the highest cumulative germination percentage (51%) in *Terminalia sericea* and appears to be the most feasible method for small scale farmers than use of sulphuric acid. Soaking in hot water for 15 and 20 min and in concentrate H_2SO_4 for 3 and 4 h gave 0 % germination.

Nicking and sand paper scarification resulted in significant increase in shoot length (9 and 11% respectively) and seedling dry weight (17 and 15% respectively) while, root length significantly decreased

(9 and 22% respectively) compared to control. Proportion of abnormal seedlings was higher in sand paper scarification (6%) followed by acid scarification with conc. H_2SO_4 (5.8 and 4.0% for 20 and 15 minutes respectively) as compared to control (1%). Fresh ungerminated (FUG) seed were significantly higher in acid scarification for 20 minutes (30.3%) followed by hot water for 20 minutes (25.8%), as compared to 1.3% in control. Dead seed were significantly higher in boiling water (77.3%) followed by 6.5% in hot water soaking for 20 minutes and 4% in acid scarification for 20 minutes. With the increase in duration of soaking in hot water or acid, the FUG and dead seed increased.

Effect of ageing: Evaluation of seed for germination at different age revealed that highest normal seedlings were observed in two year old seed (70.5%) and lowest in fresh seed (6.5%). The proportion of hard seed was higher (90.3%) in fresh seed and it decreases up to 8% in four year old seed. Hard seeds were further subjected to TZ-test for evaluating proportion of viable seeds. Among the hard seed, highest viable seed were recorded in fresh seed (97.4%) and lowest in the four years old seed (50.4%). Seedling length and dry weight was recorded higher in two year old seed and it decrease with age of seed (Table 2). Mullick and Chattergi (1967), and Hall (1985) reported that germination of *C. ternatea* seeds improved with increase in the period of storage. Lawrence *et al*. (2012) examined the hard seed breakdown treatments in six tropical pasture legumes. It was reported that fresh hard seeds of the varieties were placed in mesh bags at a field site and hard seed was measured periodically. After the first year, 64% of cv. Marc and 46% of seeds of cv. Q 9153 of *Desmanthus virgatus* remained hard; 18% of seeds of Burgundy Bean and 16% of seeds of Siratro cv. Aztec remained hard, while in *C. ternatea* only 2% and in *Centrosema* cv. Cardillo only 1% of hard seeds remained.

Table 1. Effect of physical and chemical pre-treatments on hardseededness and seedling parameters in *C. ternatea*

Treatment	Normal seedling %	Abnormal seedling %	Hard seed %	FUG seed %	Dead seed %	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)
Control (no treatment)	6.5 (14.76)	1.0 (5.74)	90.3 (71.84)	1.3 (6.44)	1.0 (5.74)	11.2	5.0	15.6
Nicking	90.0 (71.59)	4.0 (11.50)	0.0 (0.0)	4.0 (11.50)	0.0 (0.0)	12.6	5.1	19.2
Sand paper scarification	70.5 (57.11)	6.0 (14.16)	10.0 (18.43)	14.0 (21.97)	1.5 (6.94)	12.4	3.9	18.3
Hot water (80 °C) 5 min. soaking	7.3 (15.60)	0.3 (1.44)	82.3 (65.10)	8.8 (17.23)	1.5 (6.94)	12.8	4.4	17.5
Hot water 10 min.	9.3 (17.65)	0.8 (4.31)	79.5 (63.10)	9.0 (17.45)	1.5 (6.94)	13.3	3.9	17.1
Hot water 15 min.	10.0 (18.43)	1.3 (5.50)	76.3 (60.84)	10.3 (18.67)	2.3 (8.65)	12.3	3.6	15.8
Hot water 20 min.	12.3 (20.48)	2.3 (8.59)	55.8 (48.31)	25.8 (30.49)	6.5 (14.72)	11.5	3.5	14.4
Boiling water (100 °C) 5 min.	0.0 (0.0)	0.0 (0.0)	5.3 (13.28)	17.5 (24.71)	77.3 (61.52)	0.0	0.0	0.0
Conc. H_2SO_4 5 min. soaking	41.0 (39.82)	1.0 (5.74)	44.5 (41.84)	12.0 (20.26)	1.5 (6.94)	13.4	3.6	16.9
Conc. H_2SO_4 10 min. soaking	71.3 (57.58)	3.3 (10.37)	11.0 (19.36)	17.6 (24.78)	1.8 (7.67)	12.9	3.9	15.4
Conc. H_2SO_4 15 min. soaking	61.5 (51.65)	4.0 (11.50)	7.0 (15.32)	24.8 (29.83)	2.8 (9.58)	10.2	3.7	14.3
Conc. H_2SO_4 20 min. soaking	56.3 (48.59)	5.8 (13.84)	3.8 (11.19)	30.3 (33.38)	4.0 (11.50)	8.5	3.4	10.7
SEM	0.50	1.02	0.58	0.57	0.69	0.4	0.2	0.3
CD (P<0.01)	1.92	3.92	2.23	2.21	2.67	1.4	0.8	1.1

Values in parenthesis are arcsine transformed values

Table 2. Ageing effect on seed quality parameters in *C. ternatea*

Age of seed	Normal seedling % (a)	Hard seed % (b)	Germi nation (%) (a+b)	Live seed out of hard seed (%)	Abnormal seedling %	FUG seed %	Dead seed %	Seedling length (cm)	Seedling dry weight (mg)
< 3 months	6.5 (14.76)	90.3 (71.83)	96.8 (79.70)	97.4 (80.72)	1.0 (5.74)	1.3 (6.43)	1.0 (5.74)	16.5	15.6
1 year	23.3 (28.81)	72.0 (58.06)	95.3 (77.56)	90.1 (71.66)	2.3 (8.59)	1.5 (9.94)	1.0 (5.74)	17.2	15.7
2 year	70.5 (57.10)	20.3 (26.74)	90.8 (73.31)	67.2 (55.06)	3.8 (11.19)	2.5 (8.99)	3.0 (9.91)	18.9	17.0
3 year	62.5 (52.25)	16.5 (23.92)	79.0 (62.73)	60.9 (51.30)	7.5 (15.89)	7.8 (16.19)	5.8 (13.89)	17.4	16.5
4 year	56.3 (48.59)	8.0 (16.42)	64.3 (53.28)	50.4 (45.23)	11.3 (19.60)	11.3 (19.59)	12.3 (20.50)	16.2	13.9
SEM	0.93	0.69	1.75	-	0.42	0.69	1.24	0.6	-
CD (P<0.01)	3.89	2.90	7.27	-	1.73	2.89	5.17	2.3	NS

Values in parenthesis are arcsine transformed values

The correlation between age, germination and hard seed shows that two years old seed exhibit high proportion of normal seedlings (70.5%) while, hard seed reduced to 20.3% (Fig. 1).

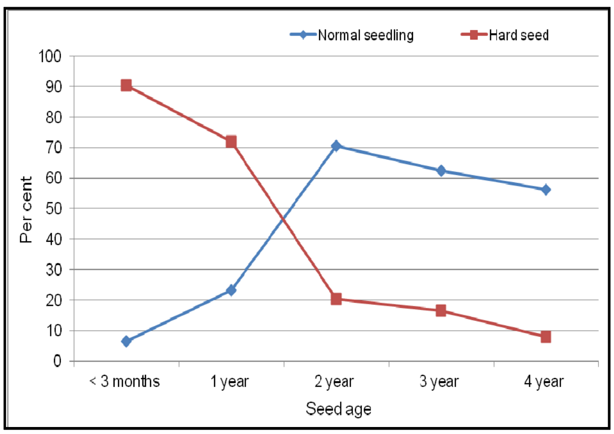

Fig 1. Effect of seed age on normal seedling and hard seed per cent in *C. ternatea*

Conclusion

Dormancy in *C. ternatea* is due to presence of hard seed coat. Nicking is an effective method to remove the hardseededness. Physical scarification methods had less adverse effect on seed germination, seedling growth and dry weight than acid scarification. Two year old seed may be used for sowing without any treatment, for achieving optimum field stand of *C. ternatea*. The suggested methods of pre-treatment can only be applied for removing hardseededness of legumes in small quantity but for the rapid treatment of large quantity of seed for commercial purpose appropriate mechanical device is to be developed which can give predictable results for a variety of seed types. Further research is also required on storability of scarified seeds.

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