



Yüzüncü Yıl Üniversitesi  
Tarım Bilimleri Dergisi  
(YYU Journal of Agricultural Science)

<http://dergipark.gov.tr/yyutbd>



Araştırma Makalesi (Research Article)

**Pretreatment Effect on Amelioration of Seed Germination of Zollinger's Indigo  
(*Indigofera zollingeriana* Miq.)\*\***

Siti MAESAROH<sup>1\*</sup>, Nurdan ŞAHİN DEMİRBAĞ<sup>2</sup>

<sup>1,2</sup>Departement of Field Crops, Faculty of Agriculture, Ankara University, Ankara, Turkey

<sup>1</sup><https://orcid.org/0000-0003-1024-284X> <sup>2</sup><https://orcid.org/0000-0002-8345-1768>

\*Corresponding Author e-posta: [maesaroh@ankara.edu.tr](mailto:maesaroh@ankara.edu.tr)

**Article Info**

Received: 23.10.2019  
Accepted: 18.01.2020  
Online Published 31.03.2020  
DOI: 10.29133/yyutbd.637005

**Keywords**

Dormancy,  
Immersion,  
Legume,  
Scarification,  
Soaking.

**Abstract:** *Indigofera zollingeriana* is one of *Indigofera* species distributed in Indonesia and known as forage crop. Seed is an important factor which influences cultivation of *I. zollingeriana* due to low seed germination. The objective of the research was to determine the effect of different pre-treatment as chemical scarification (98% of H<sub>2</sub>SO<sub>4</sub>, 0.3% of KNO<sub>3</sub>, glycerine (C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>) at 70°C), hot water at 70°C with different duration of immersion or soaking and mechanical scarification using sand paper on *I. zollingeriana*'s seed germination. Pre-treatment had significant differences and improved germination percentage, germination speed index and shoot length. The positive correlation was noted between germination percentage and germination speed index.

***Indigofera zollingeriana*'nın Tohum Çimlenmesinde Ön İşlem Etkisinin İyileştirilmesi**

**Makale Bilgileri**

Geliş: 23.10.2019  
Kabul: 18.01.2020  
Online Yayınlanma 31.03.2020  
DOI: 10.29133/yyutbd.637005

**Anahtar kelimeler**

Dormansi,  
Daldırma,  
Baklagil,  
Aşındırma,  
İslatma.

**Öz:** *Indigofera zollingeriana*, Endonezya'da dağılım gösteren ve yem bitkisi olarak bilinen *Indigofera* türlerinden biridir. Endonezya'da *I. zollingeriana*'nın yetiştiriciliğini etkileyen en önemli faktör, tohumundaki düşük çimlenme oranıdır. Bu çalışmada, *I. zollingeriana* tohumlarında, farklı sürelerde kimyasal uygulamalarının (%98'lik H<sub>2</sub>SO<sub>4</sub>, %0.3'lük KNO<sub>3</sub>, 60°C'lik C<sub>3</sub>H<sub>8</sub>O<sub>3</sub> çözelti), 70°C'lik sıcak su ve zımpara kâğıdı ile tohumu mekanik olarak aşındırma şeklinde yapılan çimlendirme ön uygulamalarının etkilerinin belirlenmesi amaçlanmıştır. Araştırma sonuçlarına göre; ön uygulamalarda çimlenme yüzdesi, çimlenme hızı endeksi ve sürgün uzunluğu bakımından önemli farklılıklar gözlenmiş olup, incelenen parametrelerde aynı zamanda artış da tespit edilmiştir. Çimlenme yüzdesi ile çimlenme hızı endeksi arasında pozitif korelasyon saptanmıştır.

\*\*This research is a part of master thesis conducted by Siti MAESAROH.

**1. Introduction**

*Indigofera zollingeriana* is one of the widely used forage plant species used in Indonesia due to high quality forage production and adaptability to drought stress under tropic conditions (Abdullah, 2010; Herdiawan and Sutedi, 2012). The plant has protein rich leaves with low tannin, lignin and cellulose contents, utilisable fibre (NDF and ADF) and high dry matter digestibility (Abdullah, 2010).

Palupi (2015) reported that top leaf meal of *I. zollingeriana* contain 28.98% crude protein, 3.30% crude fat, 8.49% crude fiber, 0.52% calcium and 0.34% phosphorus. Dianita (2012) noted interaction of arbuscular mycorrhizal fungi (FMA) had important role for transferring phosphorous and nitrogen in *Indigofera*.

Low seed viability influence plant growth and affect plant production and multiplication. Most (<85%) of the legumes have hard or impermeable seed coat (Guppy, 1912). Harrington (1916) observed that most of this is physical dormancy caused by hard seed coat that results in slow germination and development of plants. Mechanical or chemical scarification can break seed coat where metabolic inhibitor of seed is located (Copeland and McDonald, 1999). The seed condition (quality) is one of particular importance to plant cultivation in agriculture sector that rely on seed germination.

Abdullah (2014) has further reported that seed storage over 4 weeks on *I. zollingeriana* could result in decreases seed germination by 24%. Abdullah et al. (2016) has observed that long storage period affected hypocotyl length of the germinated seeds and increased mold contamination. The researcher conceived that these factors lead to poor germination of *I. zollingeriana*.

The study aimed to factors affecting seed dormancy and improves seed germination of *I. zollingeriana*.

## 2. Material and Methods

The seeds were collected from Prof. Dr. Luki Abdullah of the Department of Nutrition Science and Feed Technology, Bogor Agricultural University, Indonesia. Randomly selected healthy 3 months old yellow-brown coloured uniform seeds were selected for the experiment. Hundred (100) seed weight was measured (ISTA, 1966) and then moisture content of the seeds was determined using International Rules for Seed Testing (ISTA, 2003).

### 2.1. Pre-treatment and germination on *I. zollingeriana* seed

Eighteen pre-treatment with a control (untreated seed) were conducted on 75 seeds each of *I. zollingeriana*. The seeds were scarified using 98% of H<sub>2</sub>SO<sub>4</sub> for 5, 10, 15 and 20 min. The seeds were also scarified with 0.3% KNO<sub>3</sub> (Potassium nitrate) for 12, 24, 36 and 48 h and C<sub>3</sub>H<sub>8</sub>O<sub>3</sub> (glycerine) at 60°C for 30, 60, 90 and 120 min. All chemically scarified seeds were rinsed in running tap water for 5-10 min to remove traces of the treated chemicals until they were safe to culture for germination. Similarly, the seeds were mechanically scarified with sand paper using two and four scratches and treated with hot water at 70°C for 30, 60, 90 and 120 min.

Each of twenty five treated seeds was placed in Petri dishes lined with filter papers containing 10 ml of distilled water. Then, the seeds were cultured in growth cabinet at 24±1°C under dark conditions.

### 2.2. Measurement

Germination was observed daily until 14 days of germination (ISTA, 2007) and the seeds with 2 mm radicle length were counted as germinated. Growth rate was measured by taking length of 5 randomly selected seedlings. The germination parameters included germination percentage following Bewley and Black (1994).

$$GP (\%) = \frac{\sum ni}{N} \times 100\% \quad (1)$$

Where, GP (%) is the germination percentage, “n” is the number of normal germinated seeds at “i” day, and N is the total number of incubated seeds per test.

Speed germination was calculated by Germination Speed Index (GI), N is the number of normal germinated seeds at day “i” and D is day of count at day “i” n following Maguire (1962) and Gupta (1993) on the last day of count (7 days).

$$GI = \sum_{i=1}^n \frac{Ni}{Di} \quad (2)$$

## 2.3. Statistical analysis

The experiment was designed in a Completely Randomized Design (CRD) with 3 replications. The collected data expressed in percentage were Arcsine transformed (Steel and Torrie, 1980) before analysis of variance. The difference among means was compared by using Duncan's Multiple Range Test (DMRT). Correlation among germination parameters were analyzed using Pearson Correlation coefficient.

## 3. Results and Discussion

### 3.1. Seed weight and moisture content

Hundred seed weight was measured as 0.67 g at seed moisture content of 6.38% and this moisture content was noted for safe storage period on orthodox seeds in agreement with Roberts (1973). These seeds were subjected to seed germination experiments. *I. zollingeriana* which has hard seed coat can protect embryo during storage. Advantages of hard and thick seed coats in legume orthodox seeds are preservation of low level of metabolism by prohibiting moisture and oxygen during storage and embryo protection against mechanical damage during collection and storage (Bonner, 2008). Copeland and McDonald (1999) observed that sensitivity of large-seeded legumes and damage could be reduced > 15% seed moisture. They also noted that the threshold moisture content for small-seeded legume is 8 to 10%.

### 3.2. Seed Germination

The result show that there was a significant ( $p < 0.05$ ) different effect of pre-treatments on seed germination of *I. zollingeriana* (Table 1). The range of germination percentage was 4-78.67% after 14 days. Seed treated by sandpaper, sulphuric acid and potassium nitrate showed improvement in seed germination. The highest seed germination percentage of 78.67% and 76% were obtained on sandpaper scarified seeds after 2 and 4 scratches compared to control group and other pre-treatments. The effectiveness of sandpaper scarification was similar to Hassen et al. (2004) who noted improvement of seed germination by sandpaper scarification in 6 accessions of *Indigofera* (*I. cryptantha*, *I. brevicalyx*, *I. arrecta*, *I. spicata*, *I. trita* and *I. spicata*). In other studies Uzun and Aydin (2004) and Okunlola et al. (2011) emphasized the effectiveness of sandpaper scarification that showed high germination percentage on forage legumes as *Medicago* and *Trifolium* and African locust bean.

Sandpaper scarification by hand promoted the reduction on hard layer seed coats of *I. zollingeriana*. Similarly, mechanical scarification using sandpaper overcome physical dormancy due to hardness of seed coat by removing a thick palisade layer of macrosclereid cells characteristic of *leguminosae* related to water impermeability (Yildiztugay and Kucukoduk, 2012; Smykal et al., 2014; Chaves et al., 2017). However, some germinated seeds of *I. zollingeriana* appeared with root damage marked by black root tip. It is assumed that characteristics phenolic compounds in root cells got oxidized on contact with air and resulted in damage of the root tips in agreement with Kefeli et al., 2003, He et al., 2009. Therefore, care is needed during scratching of seeds to avoid damage to radicles. Careless scratching could result in damage to seeds or seed radicle micropylar regions fully or partially in agreement with Schmidh (2002) and Chaves et al. (2017).

The second highest germination percentage was in range 38.67-52% on seeds scarified with sulphuric acid. The effectiveness of sulphuric acid cracking and softening seed coats is in agreement with Missanjo et al. (2013) and Olatunji et al. (2013) who studied effect of pre-treatments on legumes seed germination. The seed germination after sulphuric acid scarification was lower compared to mechanical scarification with sandpaper. This could be due to long duration of immersion or high concentration of sulphuric acid that leads to embryo damage of a large quantity of seeds depending on resistance of inner integument. Missanjo et al. (2013) and Asaadi et al. (2015) also confirmed sulphuric acid was effective to improve germination percentage but they noted there was injury on embryo structure.

Three of  $KNO_3$  durations treatments had germination percentage in range of 14.67-25.33%. These results are not in agreement with previous studies where potassium nitrate has been reported to

break seed dormancy and promote seed germination (Gashi et al., 2012) on *Romonda nathaliae*. Yucel (2000) reported potassium nitrate was specified as growth-regulating substance in *Salvia* species. It is conceived that the difference in germination percentage could be due to poor compatibility of the seeds, long duration of treatment period or concentration of potassium nitrate that have not significant effects on germination percentage of *I. zollingeriana*. Sarihan et al. (2005) and Gashi et al. (2012) noted that the appropriate concentration of  $KNO_3$  improved germination seed on *Plantago lanceolata* L. and *Romando nathaliae*. Atalay et al. (2011) reported that <24 h treatment with 0.5 % potassium nitrate had negative effects on seed germinability and caused decreased seed germination of *Triticum aestivum*.

The hot water treatment with water at 70°C was not effective to improve germination of *I. zollingeriana*. The seed germination ranged 4-13.33%. Contrarily, Hassen et al. (2004) reported the effectiveness of 93°C of boiled water on germination of *I. arrecta*, *I. vohemarensis* and *I. trita* that showed improved germination percentage without remarkable risk on seed mortality. Although long duration of soaking of *I. zollingeriana* softened hard seed coats and also increased number of dead seeds due to embryo or cotyledon damage showed by extremely soft seed with fungal growth in agreement with McIvor and Gardener (1987), McDonnell et al. (2012) and Ghadir et al. (2012). These researchers confirmed that water temperature and soaking periods play an important role in seed germination.

Treatment with glycerine at 60°C resulted in seed germination range of 5.33-12% that was lower than before mentioned seed germinations. It could be due to by low temperature of glycerine that did not induce seed coat cracking. The boiling point of pure glycerine is 290°C at 760 mm (atmospheric pressure) (Anonymous, 1990). Wycherley (1960) and Schmidt (1961) emphasized use of warm treatment as glycerine was more effective to accelerate seed germination especially on some legumes. Moreover, all pre-treatment works by removing inhibitors like hard seed coat induced water and gas permeability was not much effective in this case and poorly promoted seed germination in accordance with Copeland and McDonald (1999).

There was significant difference ( $p < 0.05$ ) on germination speed showed by germination speed index value (Table 1). The result showed that sandpaper scarification, sulphuric acid scarification and potassium nitrate immersion improved germination speed with 4.49-24.36 germination index indicated seed quickly germinate and physiological quality. Seeds treated by specific duration on some glycerine and hot water had higher germination index than control group but the difference between the two was no significant. The results are similar to Rusdy (2015) who reported that sulphuric acid immersion and sand paper scarification increased germination speed on legume *Centrocrema pubescens*. Germination index was positively correlated to germination percentage with  $r = 0.964$  (Table 2). The results are in agreement with Santos (2010). Doni et al. (2014) reported that hydro treatment could increase seed germination percentage and germination speed of *Oryza sativa*. Gairola et al. (2011) and Abiri et al. (2016) reported that there was a significant positive correlation between germination percentage and germination speed on legume *Jatropha curcas* and six *indica* rice cultivars. They also reported that vigor of stored seed declines before seeds lose their ability to germinate (Delouche, 1965; Shaban, 2013).

The maximum root length was noted on the seeds germinated after 0.3%  $KNO_3$  treatment for 48 h (Table 1). It is similar to Taheri et al. (2014) who reported the effectiveness of  $KNO_3$  to improve root length of *Punica granatum* due to faster emergence of roots and shoots. There were no significant differences among some pre-treated seeds in agreement with Karaguzel et al. (2004) and Gunes et al. (2013), who noted pre-treatment had no significant effect on roots of *Lupinus varius* and *Ceratonia siliqua* root length. Potassium has substantial effect on water relation (osmotic adjustment and turgor regulation) stomatal movement, enzyme activation, photosynthesis, protein synthesis in plants (Marschner, 1995). Bhandal and Malik (1988) reported that more than 50 enzymes are activated by  $K^+$ . Thus, optimum levels  $K^+$  can improve various enzymatic processes in the cytoplasm. Therefore, it was suggested that optimum concentration of  $K^+$  at the cellular level might have acted as contributory factor for root growth and had reduced salt induced oxidative stress with lesser cell damage. This might had resulted in improved root length on the germinating seedlings in agreement with Shen et al. (2000).

Table 1. Effect of Pre-Treatment on Seed Germination and Seedling Growth of *I. zollingeriana*

Pre-Treatment	Germination Percentage (%)	Germination Speed Index	Root Length (cm)	Shoot Length (cm)
T0 Control	13.33 <sub>cde</sub>	1.76 <sup>efgh</sup>	1.9 <sub>abcde</sub>	1.5 <sub>f</sub>
T11 95-98% H <sub>2</sub> SO <sub>4</sub> 5 min	52.00 <sub>b</sub>	12.42 <sup>b</sup>	1.9 <sub>abcde</sub>	3.5 <sub>cdef</sub>
T12 95-98% H <sub>2</sub> SO <sub>4</sub> 10 min	38.67 <sub>b</sub>	9.47 <sup>bc</sup>	1.8 <sub>abcde</sub>	3.7 <sub>bcede</sub>
T13 95-98% H <sub>2</sub> SO <sub>4</sub> 15 min	45.33 <sub>b</sub>	9.74 <sup>bc</sup>	1.8 <sub>abcde</sub>	3.4 <sub>de</sub>
T14 95-98% H <sub>2</sub> SO <sub>4</sub> 20 min	40.00 <sub>b</sub>	8.31 <sup>bc</sup>	1.6 <sub>be</sub>	3.4 <sub>de</sub>
T21 0.3% KNO <sub>3</sub> 12 h	14.67 <sub>cde</sub>	4.49 <sup>de</sup>	1.8 <sub>abcde</sub>	5.0 <sub>abcd</sub>
T22 0.3% KNO <sub>3</sub> 24 h	25.33 <sub>c</sub>	10.33 <sup>b</sup>	2.1 <sub>abcd</sub>	5.2 <sub>abc</sub>
T23 0.3% KNO <sub>3</sub> 36 h	22.67 <sub>cd</sub>	8.20 <sup>bc</sup>	1.9 <sub>abcde</sub>	5.4 <sub>ab</sub>
T24 0.3% KNO <sub>3</sub> 48 h	13.33 <sub>cde</sub>	5.33 <sup>cd</sup>	<b>2.6<sub>a</sub></b>	5.5 <sub>ab</sub>
T31 60°C C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> 30 min	12.00 <sub>def</sub>	3.65 <sup>def</sup>	2.5 <sub>ab</sub>	4.4 <sub>abcde</sub>
T32 60°C C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> 60 min	8.00 <sub>ef</sub>	1.84 <sup>efg</sup>	1.7 <sub>abcde</sub>	4.9 <sub>abcd</sub>
T33 60°C C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> 90 min	10.67 <sub>ef</sub>	1.88 <sup>efg</sup>	1.4 <sub>de</sub>	3.1 <sub>ef</sub>
T34 60°C C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> 120 min	5.33 <sub>ef</sub>	1.12 <sup>fgh</sup>	2.1 <sub>abc</sub>	3.1 <sub>ef</sub>
T41 70°C hot water 30 min	4.00 <sub>f</sub>	0.18 <sup>h</sup>	1.6 <sub>bcede</sub>	3.1 <sub>ef</sub>
T42 70°C hot water 60 min	9.33 <sub>ef</sub>	2.48 <sup>defg</sup>	2.4 <sub>abc</sub>	<b>5.6<sub>a**</sub></b>
T43 70°C hot water 90 min	13.33 <sub>cde</sub>	1.27 <sup>fgh</sup>	1.8 <sub>abcde</sub>	4.1 <sub>abcde</sub>
T44 70°C hot water 120 min	9.33 <sub>ef</sub>	0.92 <sup>gh</sup>	1.6 <sub>bcede</sub>	3.4 <sub>de</sub>
T51 Sandpaper 2 scratch	<b>78.67<sub>a</sub></b>	<b>22.31<sup>a</sup></b>	1.1 <sub>e</sub>	3.3 <sub>de</sub>
T52 Sandpaper 4 scratch	<b>76.00<sub>a</sub></b>	<b>24.36<sup>a</sup></b>	1.6 <sub>bcede</sub>	3.8 <sub>bcede</sub>

Means followed by the same letter within a column differ not significantly at 5% probability

Pre-treatment has significant ( $P < 0.05$ ) different effect in germinated seed of *I. zollingeriana* that increased shoot length more than control group in range 3.3-5.6 cm after 14 days. It might have been caused by increasing quick seed germinated lead to early photosynthesis in agreement with Mabhaudhi (2009). There were no significant correlation between germination percentage and both of root length and shoot length (Table 2).

Table 2. Pearson correlation coefficient matrix between some germination and vigour test parameters on *I. zollingeriana* seed.

Correlation	Germination Index	Shoot Length	Root Length
Germination Percentage	0.964	-0.169	-0.452
	0.000*	0.488 <sup>ns</sup>	0.052 <sup>ns</sup>
Germination Speed Index		-0.004	-0.35
		0.988 <sup>ns</sup>	0.142 <sup>ns</sup>

\* ns : significant and non-significant at 5% probability

#### 4. Conclusion

Different pre-treatment had different effects and promoted germination on *I. zollingeriana* seed showed by the same germination parameters. Scarification by sandpaper could be an alternative to break hard seed coat of *I. zollingeriana* in laboratory scale. Appropriate pre-treatment can improve germination parameters. Therefore, the easy, low cost and quick pre-treatment's data obtained from laboratory study can be a recommended for faster *ex situ* germination and nursery establishment.

#### Acknowledgements

The authors are grateful to Prof. Luki Abdullah, Bogor Agricultural University, for help in give of seeds.

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