



## A Study on Germination Biology of Wild Mustard (*Sinapis arvensis* L.)

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### ARTICLE INFO

Research Article

Received : 10/11/2020

Accepted : 10/01/2021

Keywords:

Wild mustard  
*Sinapis arvensis*  
Dormancy  
Seedling  
Germination

### ABSTRACT

This study has been carried out in 2017-2018 in order to determine seed dormancy and effective germination depth wild mustard (*Sinapis arvensis* L.). The in-vitro dormancy breaking experiments (tip breaking, sanding, H<sub>2</sub>SO<sub>4</sub> application, holding in flowing and still water, GA<sub>3</sub>, KNO<sub>3</sub> and GA<sub>3</sub>+KNO<sub>3</sub> combination application) has been applied to wild mustard seeds collected from wheat field in Tokat province and has been applied to wild mustard seeds collected from wheat field in Tokat province and the most effective method was determined as 1000 ppm GA<sub>3</sub>+KNO<sub>3</sub> with 98% impact on seed germination at 15°C within 72 hours. In contrast germination rate has been calculated as 5% in control plants. Furthermore 15°C was assessed as optimum temperature for seed germination was the most effective temperature and during depth studies 100% of wild mustard seeds germinated at 3-5 cm. Because of the difficulties with the work with seeds and plants that have dormancy, these data will contribute future studies.

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## Introduction

Weeds, that grow in undesired areas, have harmful effect on crop plants and other living things (Özer et al., 2003). Wild mustard (*Sinapis arvensis*) is an annual plant which damage mainly cereals and field crops as well as several other plants. Wild mustard plant prefer irrigated areas for growth and dense infestations can be found in fields, orchards and pastures (Uygur et al., 1986; Özer et al., 1999).

Weeds compete with crop plants and cause significant yield losses. Weed seeds may contaminate by mix into harvested products and whole plants may contain poisonous substances like mustard oil allyl isothiocyanate. These are harmful to animal and human health (Cooper and Johnson, 1984; Özer et al., 2003; Direk and Gül, 2003; Güncan and Karaca, 2010). Economic damage threshold of mustard during early stage of wheat has been demonstrated as 0.1 plant/m<sup>2</sup> in several studies (Anonymous, 2017). In one of the studies of Başaran and Kadioğlu (2016) economic damage threshold in Tokat has been estimated as 0.67-1.37 plant/m<sup>2</sup>. On the other hand, Şin et al. (2016) have carried out a study to identify weeds that mix into harvested wheat in Tokat province and observed intensely mix of weed into wheat. In addition, herbicide resistant wild mustard populations have been detected in several

studies conducted in different parts of Turkey (Topuz, 2007; Gürbüz, 2016; Şin and Kadioğlu, 2019).

As a result of damage to crop plants weed management has become a priority. It is extremely important to know the biology and ecological requirements of weeds for proper management (Serim and Sözeri, 2011; Şin et al., 2018). This study has been carried out to determine seed dormancy, dormancy breaking methods and optimum germination depths of *Sinapis arvensis* seeds.

## Material and Method

### Material

Wild mustard seeds used in this study have been collected in 2017 from wheat fields in Tokat Gaziosmanpaşa University experimental area and Tokat Kazova. Mature mustard beans have been collected from plants and seeds have been separated through sieving, labeled and stored at dark under laboratory conditions. During study 9 cm diameter glass petri dishes containing filter papers, incubators, dormancy breaking chemicals (Gibberellic acid (GA<sub>3</sub>), Potassium nitrate (KNO<sub>3</sub>), Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>)), nail scissor and sandpaper No:0 have been used.

### Method

#### Determination of Optimum Germination Temperature of Wild Mustard (*S. arvensis* L.)

An experiment was arranged to find optimum germination temperature of freshly collected wild mustard seeds. Studies have been carried out in 9 cm petri dished and 2-fold Whatman filter paper were placed inside each dish. A total of 25 seeds were placed into each petri dish and about 3 ml sterile water was added to initiate germination. The petri dishes have been stored in incubator at 5, 10, 15, 20, 25, 30 and 35°C and 16/8 (day/night) for 30 days. The experiment has been arranged as randomised plot design with 4 replicates and 2 repeats. The germination has been approved when the plant length reached to 0.5 cm (Uygur and Koch, 1990; Üremiş and Uygur, 1999).

#### Dormancy Breaking Studies in Wild Mustard (*S. arvensis* L.)

Dormancy breaking studies of wild mustard (*S. arvensis*) seeds have been performed based on standard dormancy breaking methods of ISTA (2016). 3 months old wild mustard seeds were used in the experiment. The following methods have been used;

- Soak in Sulphuric acid ( $H_2SO_4$ ) (15, 30, 45, 60, 90, 120 sec., 3, 5, 10, 15 min),
- Tip breaking method
- Sand application
- Folding method (7, 15, 30, 50 and 60 days)
- Soak in still water (24, 48 and 72 hours)
- Soak in flowing water (24, 48 and 72 hours)
- Gibberellic acid ( $GA_3$ ) application at different doses (500, 750, 1000, 1500 and 2000 ppm) and with different methods (dipping out, soak and keep in solution, apply with irrigation water).
- Potassium nitrate ( $KNO_3$ ) application
- Gibberellic acid ( $GA_3$ ) and Potassium nitrate ( $KNO_3$ ) application
- Non applied control

The experiment has been arranged as randomised plot design with 4 replicates and 2 repeats. The germination studies have been carried out in 9 cm petri dished and 2-fold Whatman filter paper were placed inside each petri dish in order to create humidity. About 3 ml sterile water was added to each petri dish to initiate germination. During experiments 20 seed were placed into each petri dish. Petri dishes have been stored at 15°C and 16/8 (day/night) for 30 days. Seeds have been considered to be germinated when the radicle was 2 mm in length. After the applications, the results were subjected to Tukey test with the SPSS package program and the differences were revealed according to the  $P \leq 0.05$  significance level.

#### Sulphuric Acid Application ( $H_2SO_4$ )

The first step of the study was to arrange the homogeneity of collected seeds by selection. Before the sulphuric acid application, the selected seeds were separated by 150 for each application and subjected to 96% sulphuric acid ( $H_2SO_4$ ) for 15, 30, 45, 60, 90, 120 sec., 3, 5, 10, 15 minutes. After applications seeds were sieved through iron sieve and washed totally with tap water.

#### Tip Breaking Method

In this method small cutting was opened on seed coat with nail scissor without damaging embryos. Cutted seeds were transferred to incubator and stored.

#### Sanding Method

Sanding, a method that is generally applied to plants with thin seed coat, have been used in this study to assess the impact of seed coat on dormancy. Using water sandpaper, no=0 wild mustard seed coats were scratched in order to evaluate the effect of coat.

#### Folding Method

Folding have been applied in order to determine the vernalisation requirement of wild mustard seeds. In this method wild mustards seeds have been placed on wetted filter papers and covered with another and then moistured with water to create humidity. This humid growth areas have been stored at +4°C in the dark for 7, 15, 30, 50 and 60 days, then -18°C for 7 and 15 days and at last kept in incubator for 30 days.

#### Soak in Still Water

Due to difficult permeability of seed coats the wild mustard seeds have been soaked in distilled water for 24, 48 and 72 hour and at the end of this period seeds have been sieved in order to discard water and then kept on sterile filter paper.

#### Soak in Flowing Water

Some plant seeds contain chemicals which prevent seed germination. A study has been carried out with mustard seeds to evaluate if the germination was prevented by this kind of substances. On this purpose wild mustard seeds have been soaked in to flowing water for 24, 48 and 72 hours.

#### Gibberellic Acid ( $GA_3$ ) Application

Gibberellic acid ( $GA_3$ ) is responsible for inducing embryo growth. After  $GA_3$  application endo-  $\beta$ -mannanase secretion produced in endosperm disrupts cell wall to promote germination (Yamaguchi and Kamiya, 2002). In our study 3 ml of  $GA_3$  at different concentrations (500, 750, 1000, 1500 and 2000 ppm) have been applied to each petri dishes. In addition, wild mustard seeds have been treated with  $GA_3$  by dipping out for 3, 5 and 10 min. and soaking in solution for 12, 24, 48 hours. At the end of this period seeds have been sieved in order to discard water and dried. Dried seeds have been placed onto petri dishes and stored in incubator at 15°C for 1 month.

#### Potassium Nitrate ( $KNO_3$ ) Application

Potassium nitrate has the same effect with  $GA_3$  when applied to seeds. In our study 3 ml of potassium nitrate at different concentrations (500, 750, 1000, 1500 and 2000 ppm) have been applied to each petri dishes.

#### $GA_3 + KNO_3$ Combination

$GA_3 + KNO_3$  was applied at a dose of 1000 ppm by combining the dose of 1000 ppm, which obtained the best results of potassium nitrate and glyceric acid applications. The trials have been carried out with similar method of gibberellic acid applications.

#### Germination Study of Wild Mustard Seeds at Different Depths

A study was carried out to determine maximum germination depth of seeds. In this study five dormancy broken wild mustard seeds have been sowed on each cylindrical pot containing 1/3 sterile peat and soil. The seeds have been sowed at different depths including 3, 5, 7, 12 ve 15 cm and pots were kept in incubator at 15°C for 21 days. Pots with a depth of 20 cm were used in the experiment. Plants emerging on the soil surface are considered to germinate when dicotyledonous leaves are formed. The experiment has been arranged as randomised plot design with 4 replicates and 2 repeats. Emergence depths have been calculated based on arithmetic rate.

## Results and Discussion

### *Determination of Optimum Germination Temperature of Wild Mustard Seeds*

During germination temperature studies the highest seed germination have been achieved at 15°C (%5) while none of the seeds germinated at lower temperatures like 0 and 5°C as well as higher temperatures like 30 and 35°C. The germination rate at 10, 20 and 25°C have been calculated as 2%, 3% and 2% respectively. Compared to germination breaked seeds the germination of non-treated control seeds were 5%. These findings showed that wild mustard seeds have high dormancy. The same results have been obtained from different studies of researchers. Ateş and Üremiş (2018) collected wild mustard seeds from wheat fields in Şanlıurfa ve Batman and established germination temperature studies with dormancy breaked seeds. In this study the minimum, optimum and maximum germination temperatures have been found as 10, 15, 25°C, respectively.

### *Dormancy Breaking Studies*

The results of dormancy breaking studies have been given in Table 1.

### *Sulphuric Acid Application*

When Wild mustard seeds stored at 15°C 30 minutes, the germination have not been achieved at lower storage periods. The most effective result (30% germination) has been obtained from sulphuric acid application for 3 to 5 minutes. Several researchers worked on dormancy breaking with sulphuric acid. Güncan (1976) kept wild mustard seeds into concentrated sulphuric acid for 30 minutes and observed 1% to 9% germination. Erciş et al. (1993) applied concentrated sulphuric acid for 30 and 60 seconds and 3% of treated seeds germinated. Akın (2004) achieved 18% germination with sulphuric acid applied wild mustard seeds. Ateş (2017) carried a study with 12 month and 1 month stored seeds and found the highest germination (91.9%) in 60 seconds and 12 month stored seeds. In the same study burn and disruption was observed in seeds treated for 30 minutes with sulphuric acid. The impact of plant variety and duration of applications have been addressed in several other studies.

Table 1. The germination percentages of seeds after different germination breaking experiments

Application method	Time	Germination %	Tukey Grup
Sulphuric Acid Application	15 sec	0	N
	30 sec	0	N
	45 sec	0	N
	60 sec	5	M
	90 sec	10	JK
	120 sec	20	H
	3 min	30	F
	5 min	30	F
	10 min	20	H
	15 min	10	JK
Tip Breaking		10	J
Sanding		30	F
Folding (+4°C)	7 day	5	M
	15 day	5	M
	30 day	5	M
	50 day	10	JKL
	60 day	10	JK
Folding (-18°C)	7 day	2	MN
	15 day	0	N
Soak in Still Water	24 hours	0	N
	48 hours	5	KLM
	72 hours	0	N
Soak in Flowing Water	24 hours	0	N
	48 hours	5	M
	72 hours	5	LM
Giberrellic Acid (GA <sub>3</sub> ) Application	500 ppm	50	D
	750 ppm	80	B
	1000 ppm	97	A
	1500 ppm	60	C
	2000 ppm	40	E
Potassium Nitrate (KNO <sub>3</sub> ) Application	500 ppm	10	IJ
	750 ppm	15	I
	1000 ppm	25	G
	1500 ppm	20	H
	2000 ppm	10	J
Control	Pure water	5	M
1000 ppm GA <sub>3</sub> + KNO <sub>3</sub>		98	A

P<0.05

### **Tip Breaking and Sanding**

When tip breaking has been applied to wild mustard seeds germination have been calculated as 10% while the percentage have been 30% in sanding. Several studies reveal the effect of tip breaking and sanding. Ateş (2017) sanded 12 months stored seeds and observed 76% germination. In the same study the rate was 65.8% in 1 month old seeds. These results have been supported by finding that sanding promote seed germination.

### **Folding Application**

After several storage conditions germination have been observed in seeds at -18°C. On the other hand, the germination rate of seeds stored at +4°C for 7, 15, 30, 50 and 60 days have been found as 5, 5, 5, 10 and 10% respectively. Depending on these results increasing effect of longer duration period on germination have been determined.

### **Soak in Still and Flowing Water**

As seeds soaked in still and flowing distilled water at 24°C for 24, 48 and 72 hours have been assayed for germination and no effect of distilled water have been observed (Table 1). When Topuz (2007) established the same experiment with seeds resistant or susceptible to chlorosulfuron the germination percentage in resistant and susceptible populations were 71.8-75.0% and 27.1-31.2% respectively. In another study Ateş (2017) soaked seeds into distilled water for 6, 24, 48, 72, 96 and 120 hours and the germination percentage were 46.4, 26.5, 12.5, 3.5, 3.3, 2.0 respectively. In that study germination decreased parallel to duration increase.

### **Gibberellic Acid (GA<sub>3</sub>) Application**

In this study GA<sub>3</sub> at different concentrations (500, 750, 1000, 1500 and 2000 ppm) has been applied to wild mustard seeds to determine the effect on germination. The most effective concentration for germination has been found as 1000 ppm GA<sub>3</sub> (97% germination) and the decrease occurred when the concentration increased. The highest germination at 1000 ppm application has been observed in 5th day. In addition, the seeds have been soaked in 1000 ppm for 24, 48 and 72 hours as well as dipped for 3, 5 and 10 minutes but germination has not been occurred. Erciş et al. (1993), stored seeds for 6 months at room temperature and then treated seeds with H<sub>2</sub>SO<sub>4</sub> for 30 and 60 seconds and later applied 500 ppm GA<sub>3</sub>. The germination percentage was found 3% in only H<sub>2</sub>SO<sub>4</sub> application and 39% in H<sub>2</sub>SO<sub>4</sub> and GA<sub>3</sub> combination. Topuz (2007) carried out a study with chlorsulfuron resistant and susceptible seeds and applied GA<sub>3</sub> at different concentrations (0.1, 1, 5 and 10 mM). In this study the statistical differences were not observed in the point of germination. Ateş (2017) worked with 1 month and 12 months stored seeds and evaluated 2000 ppm GA<sub>3</sub> as most effective concentration. The germination percentage with 1 month and 12 months seeds has been determined as 95.7% and 100%, respectively.

### **Potassium Nitrate (KNO<sub>3</sub>) Application**

The wild mustard seeds have been treated with different concentrations of potassium nitrate (KNO<sub>3</sub>) for 30 days. The percentage in control plants have been calculated as 5% while this rate has been found as 25% in 1000 ppm. Goudey et al. (1987) used different chemicals (KNO<sub>3</sub> and NH<sub>4</sub>Cl) to break dormancy in seeds and no effect was

observed with single application of each chemical but the rate increased to 90 % in chemical combination. Uludağ and Özer (1999) treated seeds with different chemicals (GA<sub>3</sub>, KNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>) as well as applied mechanical methods (scratch) to break dormancy. In KNO<sub>3</sub> treatment the germination of *Cerastium dichotomum* L. seeds were determined as 90%. In other research Ateş (2017) applied KNO<sub>3</sub> at concentrations of 0.5, 1, 3, 4% to 1- and 12-month-old wild mustard seeds and the germination percentage at 0.5% concentration were 75.1% and 47.8% respectively

At the end of this study the ideal germination rate (97%) has been achieved in 1000 ppm GA<sub>3</sub> application and irrigation water for 5 days. In order to promote hormonal germination three methods including sanding, 1000 ppm gibberellic acid and 1000 ppm potassium nitrate+1000 ppm gibberellic acid have been applied together. Sanding+gibberellic acid has caused 92.5% decrease in the germination while potassium nitrate+gibberellic acid application resulted in 98% increase.

### **Seed Germination at Different Soil Depths**

In depth study wild mustard seeds treated with 1000 ppm GA<sub>3</sub> and KNO<sub>3</sub> for 48 hours to break dormancy have been used. The optimum germination percentage has been achieved as 100% at 3 and 5 cm depths. Parallel to increase in depth the germination has decreased. The germination decreased to 75% at 7 cm depth and at 12-15 cm seeds have not been able to emerge to soil surface.

## **Results**

Wild mustard is an annual Mediterranean plant that is distributed mainly in lentil and sugarbeet fields, gardens and pastures. The higher dormancy level of plant hardens the studies.

It is one of the plants that is difficult to study due to the high rate of dormancy in its seeds. To select the proper management method its essential to know plant biology. The optimum germination temperature of newly collected seeds having dormancy have been determined as 15 °C. During studies with different applications (soak in sulphuric acid for 3-5 minutes, sanding) the germination percentage have been not found as much as 30%. The other application had little effect on dormancy by affecting coat permeability. About 97% germination has occurred at 1000 ppm GA<sub>3</sub> application for 5 days. While 98% observed at 1000 ppm GA<sub>3</sub>+KNO<sub>3</sub> treatment for 2-3 days. The plant is economically important and the results of studies like this will promote further studies.

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