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

Bahadır Şin1,a*, İzzet Kadioğlu1,b

1Department Of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpasa University, 60250 Tokat, Turkey
2Corresponding author

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, H2SO4 application, holding in flowing and still water, GA3, KNO3 and GA3+KNO3 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 GA3+KNO3 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.

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; Dirck and Gül, 2003; Günçan and Karaca, 2010). Economic damage threshold of mustard during early stage of wheat has been demonstrated as 0.1 plant/m² 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/m². 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 seperated through sieving, labeled and stored at dark under laboratory conditions. During study 9 cm diameter glass petri dishes containing filter papers, incubators, domansy breaking chemicals (Gibberellic acid (GA3), Potassium nitrate (KNO3), Sulfuric acid (H2SO4)), 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 dishes 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; Üremis 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 standay 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$_2$SO$_4$) (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 days)
- Gibberelic 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
- Gibberelic 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 dish 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 radicule 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≤0.05 significance level.

**Sulphuric Acid Application (H$_2$SO$_4$)**

The first step of the study was to arrange the homogenisity of collected seeds by sellection. Before the sulphuric acid application, the selected seeds were separated by 150 for each application and subjected to 96% sulphuric acid (H$_2$SO$_4$) 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.

**Gibberelic Acid (GA$_3$) Application**

Gibberelic acid (GA$_3$) is responsible for inducing embryo growth. After GA$_3$ application endo- β-mannanaz 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 gibberelic 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 breaked 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 aritmetic 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 (%7) 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 broken 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 Üremen (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.

**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            |            |
| Giberrelic Acid (GA₃) Application | |   | |
| 500 ppm                    | 50   | D             |            |
| 750 ppm                    | 80   | B             |            |
| 1000 ppm                   | 97   | A             |            |
| 1500 ppm                   | 60   | C             |            |
| 2000 ppm                   | 40   | E             |            |
| 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 GA₃+ KNO₃         | 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 chlorosuphuron 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, 3.5, 2.0 respectively. In that study germination decreased parallel to duration increase.

Gibberellic Acid (GA₃) Application

In this study GA₃ 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 GA₃ (97% germination) and the decrease occured 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 of for 3, 5 and 10 minutes but germination has not been occured. Erçin et al. (1993), stored seeds for 6 months at room temperature and then treated seeds with H₂SO₄ for 30 and 60 seconds and later applied 500 ppm GA₃. The germination percentage was found 3% in only H₂SO₄ application and 39% in H₂SO₄ and GA₃ combination. Topuz (2007) carried out a study with chlorosulfuron resistant and susceptible seeds and applied GA₃ 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 GA₃ as most effective concentration. The germination percentage with 1 month and 12 months seeds have been determined as 95.7% and 100%, respectively.

Potassium Nitrate (KNO₃) Application

The wild mustard seeds have been treated with different concentrations of potassium nitrate (KNO₃) 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 (KNO₃ and NH₄Cl) 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 (GA₃, KNO₃, H₂SO₄) as well as applied mechanical methods (scratch) to break dormancy. In KNO₃ treatment the germination of Cerastium dichotomum L. seeds were determined as 90%. In other research Ateş (2017) applied KNO₃ 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 GA₃ 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 GA₃ and KNO₃ 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 dormacy have been determined as 15 ºC. During studies with different applications (soak in sulphuric acid for 3-5 minutes, sanding) the germination percentage have been 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 GA₃ application for 4 days. While 98% observed at 1000 ppm GA₃+KNO₃ treatment for 2-3 days. The plant is economically important and the results of studies like this will promote further studies.

References

Akin B. 2004. Dornamsı kırcık yöntemlerin yabanç ot tohumları üzerinde etkileri. Dumlupınar Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Kütahya.

Anonymous 2017. Buğday Entegre Mücadele Teknik Talimatları. T.C. Gıda, Tarım ve Hayvancılık Bakanlığı, Tarmsal Araştırmalar ve Politikalar Genel Müdürlüğü, Bitki Sağlığı Araştırmaları Daire Başkanlığı, Ankara, 138 sayfa.

Ateş E. 2017. Batman ve Şanlıurfa buğday alanlarında bulunan yabanı otlar ile yabanı hardal (Sinapis arvensis L.) ve kısır yabanı yulaflı (Avena sterilis L.)ın bazı biyolojik özelliklerinin belirlenmesi. Mustafa Kemal Üniversitesi / Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi.

731
Ateş E, Üremiş İ. 2018. *Sinapis arvensis* L. (yabani hardal) ve *Avena sterilis* L. (kısır yabani yulaf) tohumlarının çimlenme şacıklıklarını belirlenmesi. *International Journal of Agricultural and Natural Sciences*. Uluslararası Tarım ve Doğa Bilimleri Dergisi. E-ISSEC:2651-3617, 1(2):154-159, 2018.

Başaran B, Kadoğlu İ. 2016. Tokat İli Büğday Ekim Alanlarında Sorun Olan Yabani Hardal (*Sinapis arvensis* L.)’nın Ekonomik Zarar Eşliğinin Belirlenmesi. *Turk J. Weed Sci.*, 19(1):1-5.

Cooper MR, Johnson AW. 1984. Poisonous plants in Britain and their effects on animals and man. *Her Majesty’s Stationery Office*, London, England. 305 pp.

Direk M, Gül A. 2003. Konya ticaret borsasında buğday fiyat oluşumunu etkileyen faktörler. *Ticaret Borsası Dergisi*, Sayı: 16, Konya

Erciş A, Tastan B, Yıldırım A. 1993. Yabani hardal (*S. arvensis*)’n bazı biyolojik özellikleri üzerinde araştırması. *Türkiye I. Herboloji Kongresi* (3-5 Şubat1993 Adana) 55-61.

Günçan A. 1976. Erzurum çevresinde bulunan yabancı otlar ve önemlilerinden bazılarının yazılık hububatta mücadele imkanları üzerinde araştırması. Atatürk Üni. Yayınları No. 446. Erzurum.

Günçan A. ve Karaca M. 2014. Yabancı Otlar Mücadelesi (Güncelleştirilmiş ve İlabeli Üçüncü Baskı) Selçuk Üniversitesi Ziraat Fakültesi Yayınları Konya, 310s.

Gürbüz R. 2016. Adana İli Büğday Ekim Alanlarında ALS Inhibitori Herbisitlere Karşı Dayanıklılık Kazanmış Yabani Yulaf (*Avena sterilis* L.) ile Yabani Hardal (*Sinapis arvensis* L.) Popülasyonlarının Belirlenmesi ve Dayanıklılık Haritalarının Oluşturulması. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi, Adana. 372 sayfa.

ISTA, 2016. *Rules Proposal Fort the International Rules for Seed Testing*, 2016 edition 41s.

Özer Z, Kadoğlu İ, Önen H, Tursun N. 2003. Herboloji (Yabancı Otları). Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi Yayınları No:29. Ders Notları Serisi No:10, 579 sayfa.

Özer Z, Önen H, Tursun N, Uygur N. 1999. Türkiye’nin Bazı Önemli Yabancı Otları (Tanımları ve Kimyasal Savaşımlar). Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Yayınları No:38, Kitap Serisi No:16, 434 sayfa.

Serim AT, Sözeri S. 2011. Doğu Tarla Hazerani [Consolida orientalis (Gay) Schrödl. (Ran)]’nin Çımlenme Biyolojisi ve Araştırması. *Türkiye Herboloji Dergisi*, 14(1-2):9-16.

Şin B, Kadoğlu İ. 2019. Amasya, Çorum, Tokat ve Yozgat illerinde buğday yetiştirilen alanlarda *Sinapis arvensis* (Yabani Hardal) bitkisinin varlığının ve Tribenuron methyl etken maddeli herbisite karşı hassaslığın belirlenmesi. 3. Uluslararası UNİDOKAP Karadeniz Sempozyumu. “Sürdürülebilir Tarım ve Çevre 21-23 Haziran 2019 Tokat, Türkiye.

Şin B, Kadoğlu İ, Altuntaş G, Kekçê M, Kazankiran T. 2018. Çeti (*Prosopis faracta* (Banks&Sol.) J.F.Mac.) Bitkisinin Tohum Çımlenme Biyolojisinin Araştırılması. *Turk J. Weed Sci.*, 21(1): 53-60.

Şin B, Kadoğlu İ, Kamuşi B. 2016. Tokat İlinde Büğday Ürunü İçerisine Karışan Yabancı Otlar Tohumlarının Belirlenmesi. *Turk J Weed Sci.*, 2016:19(2): 28 – 37.

Topuz M. 2007. Marmara Bölgesinde Buğday Tarlalarda Bulunan *Sinapis arvensis* L. (Yabani Hardal)’ın Süfonylure Grubu Herbisitlerine Karşı Oluşturduğu Dayanıklılık Üzerine Araştırması. Ege Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, 202 sayfa. İzmir.

Uygur FN, Koch W, Walter H. 1986. Çukurova Bölgei Buğday, Pamuk Ekim Sistemindeki Önemli Yabancı Otların Tanımı. *PLITS*, 1986/4 (1): 196s. Vol.2, 379-384.

Uygur FN, Koch W. 1990. *Cynodon dactylon* L. Pers. ve *Sorghum halepense* (L.)Pers.’nin Tohumlarının Çımlenmesini ve rizom boğumlarının Sırmesini Etkileyen Faktörlerin Araştırılması. *Doğa- Turkish Journal of Agricultural and Forestry*, 14:192-201

Üremiş İ. Uygur FN. 1999. Çukurova Bölgesindeki Önemli Bazı Yabancı Otlar Tohumlarının Minimum, Optimum ve Maksimum Çımlenme Şacıklıkları, *Türkiye Herboloji Dergisi*, 2 (2): 1-12.

Yamaguchi S, Kamiya Y. 2002. *Gibberalins and Light-stimulated Seed Germination*. *J. Plant Growth Regul*, 20:369-376.