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Effect of Mechanical Damage on to Emergence Rate and Emergence Force of Some Wild Sunflower (*Helianthus* L. Spp.) Seeds

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Abstract

To investigate the possible use of wild sunflower species (*Helianthus* spp.) with cultural varieties at classical breeding studies and to overcome the seed dormancy and germination problems encountered at germination stage in seed, the effect of mechanical damage to the seed on to emergence rate and emergence force was determined. The study was conducted at Uludağ University, Agricultural Faculty, Field Crops Department, Plant Tissue Culture Laboratory and green house in 2014. Seeds of wild sunflower genotypes were obtained from 3 different sources (USDA-USA, Germany, Canada). The seeds imbedded in water one day were scratched carefully from embryo sites and then placed into viol containing 1 : 1 peat: soil mixture (v/v) at 24 ± 2°C in 16h/8 h (light/dark) in the growth chamber. Emergence rate and emergence force values were taken after 10 and 17 days from seeding. Emergence rate and emergence force values varied from 0 - 100% depended on the genotype. The highest emergence rate values (100%) were obtained from genotypes numbered as 4, 17, 20, 24, 37, 59 while genotypes numbered as 31, 32, 33, 38, 39, 40, 41, 46, 47, 48, 49, 51, 52, 55 did not have emergence rate values (0%). The highest emergence force values (100%) were obtained from genotypes numbered as 4, 17, 19, 20, 24, 37, 59 while genotypes numbered as 31, 32, 33, 38, 39, 40, 46, 48, 49, 51, 52, 55 did not have emergence force values (0%)

Key Words: Sunflower, *Helianthus* spp., wild type genotypes, emergence rate and emergence force

Introduction

Sunflower (*Helianthus annuus,* L) is an important healthy edible oilseed crop worldwide and the production of sunflower in Turkey has great potential as oil crop. The development of new genotypes with good agronomic performances (new types of vegetable oil, disease resistant, drought resistant etc.) is an important target in sunflower breeding programs. However the lack of genetic variation for important traits is limited in cultivated sunflower species. In general, wild *Helianthus* species are of considerable interest as a source of genetic variation for economically important characters, such as male sterility (Serieys and Christov, 2005; Christov, 2013), disease resistance (Fernandez-Martinez et al. 2010; Jan and Chandler, 1985; Seiler and Reiseberg, 1997; Tan et al., 1992), salt tolerance (Chandler and Jan, 1985), herbicide tolerance (Miller and Al-Khatib, 2004), chemical constituents (Tosun and Özkal, 2000). Wide hybridization creates new genetic variation in sunflower breeding. However, the application of new genes from wild relatives for improving the characteristics of sunflower is limited mainly by obtaining interspecific hybrids via sexual crossing due to incompatibility mechanism. It is also well known that wild sunflower species are self-incompatible and have a deep dormancy, even for freshly harvested achenes whereas modern sunflower cultivars are self compatible and have short lived seed dormancy (Chandler and Jan, 1985; Fernández-Martínez et al., 2009; Marek et al., 2004; Seiler, 1998). Therefore it is not easy germination of wild type sunflower seeds in a reproducible number and efficient way.

Seed dormancy is defined as the seed that prevents its germination even under favorable

environmental conditions. Seed presents primary dormancy after harvesting while the secondary dormancy may occur due to unfavorable environmental conditions (Şehirali, 2002; Vujaković et al., 2012). The presence of seed dormancy can cause great germination problems of wild type sunflowers (Seiler, 2010). Several physical and chemical factors (temperature, water stress, depth of sowing as the irregular shape, small seed size, moisture content and the age of achenes at harvest and seed dormancy) greatly affect seedling emergence and seedling vigor of sunflower (Connor and Hall, 1997). Dormancy is a temporary factor of growth and development, which is endogenously controlled, but also environmentally imposed (Amen, 1968; Pallavi et al., 2010; Presotto et al. 2014; Şehirali, 2002). Seed dormancy is associated with accumulation of abscisic acid (ABA) during maturity, gibberellic acid (GA_3), thickness of pericarp and seed coat permeability by many scientists (Finch-Savage and Leubner-Metzger, 2006; Le Page-Degivry et al., 1996; Subrahmanyam et al., 2002). It is known that sunflower seeds keep their germination capacity for about six days after pollination become dormant 16 days after pollination (Maiti et al., 2006) and remain dormant for 45-60 days after harvest (Jambhulkar, 1995) due to accumulation of ABA and presence of thick pericarp and seed coat (Le Page-Degivry et al., 1996). Seed dormancy problem has been solved by manipulating the date of sowing so that they have enough time to break dormancy naturally. The embryo culture is another way to reduce seed dormancy period (Chandler and Beard, 1983). In one study, it was shown that seeds removed from a head 10 to 12 days after fertilization and immediately placed in MS media for embryo growth has the capacity to germinate (Dagustu et al., 2010, Dagustu et al., 2012). Many different physical (mechanical scarification, hull removal) and chemical pre-treatments-priming (Acetone, Ethrel, Ethylene, KNO_3, GA_3 etc.) have been applied to overcome dormancy in wild sunflower species (Chandler and Jan, 1985; Corbineau and Come 2003; Maiti et al., 2006; Tan et al., 1992; Vujaković et al., 2012). Seiler (1994) reported that chemical pre-treatment (priming) of wild sunflower achenes with 1 mM solution of GA_3 almost doubled germination percentage over a non-treated control. It is also showed that seed priming with antioxidants (ascorbic acid, tocopherol and glutathione) improved hybrid sunflower seed germination and seedling growth under unfavorable germination conditions (Draganic and Lekic, 2012). Borghetti et al. (2002) informed that protease activity may be involved in removal of dormancy by ethylene and improve germination of the sunflower embryo. Several organic compounds such as acetone, chloroform, ethanol and many other compounds which have lipophilic properties, some degree of polarity and low molecular weight have the capacity to break seed dormancy (Adkins et al., 1984; Cohn et al., 1989; Corbineau and Come 2003). It has been stated that prolonged exposure of sunflower achene membranes to ethanol may change the cell membrane permeability leading to permanent change, not affected by hydration and dehydration. Similarly, priming in the KNO_3 solutions significantly improves germination of some crop species such as confectionary and oily sunflower genotypes under both low and high temperature with 12 hours treatment. Increasing germination rate of cultivated sunflower indicates that KNO_3 may influence the formation of free radicals, which in turn improve vigor (Ali, 2011). Seiler (1993) reported that the age of achenes at harvest of wild *H. annuus* and *H. petiolaris* had a significant influence on germination; the majority of germination took place by 21 days. Akinola et al. (2000) applied different seed treatments to induce germination in wild sunflower. He indicated that hot water at 80°C is effective for breaking dormancy. Seiler (1994) reported that chemical pre-treatment (priming) of wild sunflower achenes with a 1 mM solution of GA_3 almost doubled germination percentage over a non-treated control. It is

also showed that seed priming with antioxidants (ascorbic acid, tocopherol and glutathione) improved hybrid sunflower seed germination and seedling growth under unfavorable germination conditions (Draganic and Lekic, 2012). In a subsequent study, Seiler (1998) reported that the seeds of *H*. *petiolaris* treated with GA_3 enhanced germination to 60% irrespective of maturity and storage time compared with 30% in the control. It was observed that achenes harvested 20 days after flowering had greater germination than those harvested 40 days after flowering. Achenes of two wild annual sunflower species, *H. annuus* (common wild sunflower) and *H*. *petiolaris* (prairie sunflower), stored at room temperature (20 to 22°C) at a relative humidity of approximately 22% in a bell jar in the laboratory for 20 years were evaluated by Seiler (2010) for germination and viability. He showed that treating scarified wild achenes with 1 mM GA_3 for one hour increased germination of the stored wild *H*. *annuus* achenes from 13% to 88%, and *H*. *petiolaris* from 1.5% to 85%.

The aim of this research is to develop an efficient and simple protocol for breaking seed dormancy of wild type sunflower species (*Helianthus* spp.) obtained from different sources.

Materials and Methods

The 63 wild sunflower species *(Helianthus* spp.) obtained from different sources (USDA-USA, Germany, Canada) was used as plant materials (Table 1). The study was conducted at Uludağ University, Agricultural Faculty, Field Crops Department, and Plant Tissue Culture Laboratory and Green House in 2014. To break dormancy and ensure adequate germination, achenes were pre-treated with sterile distilled water for one day. The achenes were then mechanically scarified by cutting off the tip of the achene to distal cotyledon end (Jan and Chandler, 1985; Chandler and Jan, 1985; Ş. Tan, Personal communication, 2014) and transferred to 31 by 51 cm plastic multipot trays of 48 pots per tray containing sterile 1:1 peat : soil mixture (v:v). The treated seeds were sown at 3 cm depth and trays were then placed into green house and incubated for a period of 17 days. The 3-10 seeds were placed into multipots depends on availability of seed number. The multipots were irrigated regularly. The emergence force and emergence rate values were determined after 10 and 17 days respectively according to Şehirali (2002). There were not enough wild type seeds in hand therefore this experiment did not repeat again.

Results and Discussions

Although the response to the mechanical scarification treatment of this wide variety of *Helianthus* spp populations was highly variable, the mechanical scarification increased emergence rate and emergence force in many genotypes (Table 1). Emergence rate and emergence force values varied from 0 - 100% depended on the genotype. The highest emergence rate values were obtained from genotypes numbered as 4, 17, 19, 20, 24, 37, 62 (100%) while genotypes numbered as 31, 32, 33, 38, 39, 40, 46, 48, 49, 51, 52, 55 did not show emergence rate and emergence force (0%). The highest values of emergence rate were obtained from genotypes numbered as 4, 17, 24, 37, 62 (100%) while no seed emergence was seen from genotypes numbered as 31, 32, 33, 38, 39, 40, 46, 48, 49, 51, 52, 55 (0%). Genotypes numbered as 41, 47 have emergence force at 17 days.

The low germination of wild sunflower achenes may be the result of dormancy, and not due to seed degradation. All ungerminated seeds were hard coated and appeared to be viable as described in Seiler (2010) and Vujaković et al. (2012). They showed that wild type sunflower achenes stored at less than optimal conditions for long periods (20 years) were dormant and did not be discarded as dead. Therefore, it is thought that ungerminated seeds were probably viable but dormant in our study.

In this study, the scratching seeds of wild sunflower species soaked in water for overnight to overcome achene dormancy increased emergence rate and force as previously reported (Kaya et al., 2006). Presotto et al. (2014) also found parallel results that pericarp scarification increased water uptake 19% more than non-scarified achenes at wild *H. annuus* species.

This might be due to the built up inhibitors in the seeds and hulls during maturation. Maiti et al (2006) illustrated that the seed grown under drought conditions had harder coat, and more compact nucleus compared to seed produced under moisture conditions.

Table 1: Emergence rate and force values of some wild sunflower (*Helianthus* spp.) species

GENOTYPES	Seed number	Emergence rate 10th day	Emergence force 17th day
	(number)	(%)	(%)
H. annuus, Mexico (1)	10	60,0	60,0
H. annuus, USA, California (2)	10	90,0	90,0
H. annuus, Mexico (3)	10	90,0	90,0
H. annuus, Mexico (4)	10	100,0	100,0
H. annuus, USA, Colorado (5)	10	60,0	60,0
H. annuus, USA, New Mexico (6)	10	80,0	80,0
H. annuus, USA, Arizona (7)	10	70,0	70,0
H. annuus, USA, Mississippi (8)	10	80,0	80,0
H. annuus, USA, North Carolina (9)	10	90,0	90,0
H. annuus, USA, Kentucky (10)	10	90,0	90,0
H. annuus, USA, Wisconsin (11)	10	80,0	80,0
H. annuus, USA, Oklahoma (12)	10	30,0	30,0
H. annuus, USA, Texas (13)	10	80,0	80,0
H. annuus, USA, Oklahoma (14)	10	50,0	60,0
H. annuus, USA, Texas (15)	10	60,0	70,0
H. annuus, USA, Oregon (16)	10	90,0	90,0
H. annuus, USA, North Dokata (17)	10	100,0	100,0
H. annuus, USA, Wyoming (18)	10	60,0	60,0
H. annuus, USA, Kansas (19)	10	80,0	100,0
H. annuus, USA, Nebraska (20)	10	100,0	100,0
H. annuus, Canada, Saskatchewan (21)	10	80,0	80,0
H. annuus, USA, Arkansas (22)	10	70,0	70,0
H. annuus, USA, Minnesota (23)	10	80,0	80,0
H. annuus, USA, Tennessee (24)	7	100,0	100,0
H. annuus, USA, Iowa (25)	7	85,7	85,7
H. annuus, Unknown (26)	10	70,0	80,0
H. annuus, USA, Ohio (28)	10	40,0	60,0
H. anomalus, USA, Arizona (30)	7	14,3	28,6
H. anomalus, USA, Utah (31)	10	0,0	0,0
H. anomalus, USA,, Utah (32)	4	0,0	0,0
H. anomalus, USA, Nevada (33)	10	0,0	0,0
H. argophyllus, USA, Florida (34)	10	80,0	90,0
H. argophyllus, USA, Texas (35)	10	80,0	90,0
H. argophyllus, USA, Texas (36)	10	90,0	90,0
H. argophyllus, USA, Texas (37)	10	100,0	100,0
H. deserticola, USA, Utah (38)	9	0,0	0,0
H. deserticola, USA, Utah (39)	6	0,0	0,0
H. deserticola, USA, Nevada (40)	5	0,0	0,0
H. deserticola, USA, Nevada (41)	10	0,0	30,0
H. maximiliani, USA, Illinois (42)	10	70,0	70,0
H. maximiliani, USA, Montana (43)	10	40,0	50,0
H. maximiliani, USA, Manitoba (44)	10	80,0	90,0
H. maximiliani, USA, South Dokata (45)	10	70,0	70,0

Table 1: Continued

GENOTYPES	Seed number	Emergence rate 10th day	Emergence force 17th day
H. niveus subsp. *canescens*, USA, New Mexico (46)	4	0,0	0,0
H. niveus subsp. *canescens*, USA, Texas (47)	10	0,0	10,0
H. niveus subsp. *canescens*, USA, Arizona (48)	10	0,0	0,0
H. niveus subsp. *canescens*, USA, Utah (49)	10	0,0	0,0
H. petiolaris subsp. *fallax* ,USA, Texas (50)	10	50,0	60,0
H. petiolaris subsp. *fallax* , USA, Colorado (51)	10	0,0	0,0
H. petiolaris subsp. *fallax* ,USA, Arizona (52)	5	0,0	0,0
H. petiolaris subsp. *fallax* ,USA, New Mexico (53)	10	10,0	10,0
H. petiolaris subsp. *petiolaris*, USA, Texas (54)	9	33,3	55,6
H. petiolaris subsp. *petiolaris*, USA, Colorado (55)	10	0,0	0,0
H. petiolaris subsp. *petiolaris* , USA, California (56)	9	33,3	44,4
H. petiolaris subsp. petiolaris, USA, Nebraska (57)	10	60,0	60,0
H. bolanderi, Almanya (58)	10	20,0	20,0
H. argophyllus, Almanya (59)	10	10,0	30,0
H. petiolaris, Almanya (60)	10	50,0	50,0
H. annuus ssp. *lenticularis*, Almanya (61)	4	75,0	75,0
H. maximiliani, Kanada (62)	3	100,0	100,0
H. nuttalli, Kanada (63)	3	66,7	66,7

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