# BIOTIC AND ABIOTIC STRESS TOLERANCE

# EVALUATION OF SUNFLOWER GENOTYPES TO STEM ROT CAUSED BY SCLEROTINIA SCLEROTIORUM UNDER FIELD CONDITIONS

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## ABSTRACT

Stem and root rot caused by *Sclerotinia sclerotiorum* is the most important devastating disease of confectionary sunflowers in West Azarbayjan province of Iran particularly Khoy area. To evaluate reaction of confectionary and oilseed sunflowers against the disease, 76 genotypes including both types were inoculated by the pathogen at grain filing stage. The isolate 302 collected from the infected area (Khoy) and mass produced, was used for the experiment. Seven millimeter mycelial plugs of PDA medium including 3-day-old culture of the pathogen were put on injured site of the individual plants at 40 centimeter height. A small piece of wet cotton and two layers of Parafilm for maintaining moisture and fixing the fungal plug were employed for all treatments. The lesion length of inoculated stems was measured seven and 14 days post inoculation. The results of data analysis demonstrated significant differences of lesion length and single head yield between the genotypes. Line S53 with mean 63 millimeter lesion length and S6B with 13 millimeter lesion length demonstrated the most and least progress and infections, respectively. The local land races of confectionary sunflower including Shamshiri and Badami were more susceptible in comparison with Pestei ones against the disease.

**Key Words :** confectionary sunflower, *Sclerotinia sclerotiorum*, reaction.

# ADVANCES IN HOST PLANT RESISTANCE TO SUNFLOWER INSECT PESTS IN NORTH AMERICA

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## ABSTRACT

Resistance of wild (annual or perennial) sunflowers to insect pests is a recurring idea in the scientific literature. Though wild sunflowers may often be refractory to certain insect pests, recent research illustrates that cultivated sunflower germplasm also has considerable variation in susceptibility to insects, and that many opportunities exist to exploit host plant resistance in cultivated sunflowers. For the sunflower moth (*Homoeosoma electellum*), there are very high numbers of capitate glandular trichomes available in inbred lines and progress is being made in mapping the genes that determine trichome number. Though there are differences in the composition of secondary plant compounds in the glandular trichomes of wild and cultivated *H. annuus*, the contents of cultivated sunflowers retain toxicity or repellency to sunflower moth larvae. For the red sunflower seed weevil (*Smicronyx fulvus*), strong resistance in breeding material exists, but it not well understood. Development of germplasm and mapping of the weevil resistance is ongoing. Lastly, there is significant variation in susceptibility of sunflower inbreds to the banded sunflower moth (*Cochylis hospes*). While some public inbreds appear to be as refractory as previously identified cultivars, it is not clear whether resistance of inbred parents will translate into equivalent or better resistance in hybrids. Specific mechanisms of resistance are not well understood in all cases, but the traits found in resistant accessions and inbreds identified for North American insects should be applicable to some pests in other parts of the world.

**Key Words :** sunflower moth, banded sunflower moth, red sunflower seed weevil, antibiosis, glandular trichomes

# DISTRIBUTION OF PLASMOPARA HALSTEDII PATHOTYPES IN HUNGARY

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## ABSTRACT

The oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni, the causal agent of sunflower downy mildew, is one of the most damaging diseases in the world. There are several pathotypes (at least 36) of the pathogen which greatly influence both the effectiveness of fungicides and the resistance genes incorporated into new sunflower hybrids to combat this disease. The number of *P. halstedii* pathotypes is increasing rapidly. Recently, new pathotypes, 704 and 714, have been identified in Hungary, while as many as 5 races (100, 330, 700, 710, 730) were distributed before 2010 in the country. Our objectives are to continuously monitor this pathogen and identify pathotypes of *P. halstedii.* Samples were collected at 20 different sites in Hungary between 2012-2014 from sunflower hybrids containing *Pl6* resistance gene.and from volunteer plants. Examination of isolates was carried out using a set of sunflower differential lines based on the internationally standardized method for race identification of *P. halstedii*. Disease assessment was first performed based on the appearance of white sporulation on cotyledons. A second evaluation of true leaves was made on 21-day-old plants. According to our results six different pathotypes of the pathogen were determined. Pathotypes 704, 714 and 700 were the most widespread while 730 and 710 were also common during the examination period. Occurrence of a new pathotype, 734, is also suspected but not proved during the survey. Thus, continuous survey and identification of the virulence phenotype of *P. halstedii* is essential for sustainable sunflower breeding and plant protection.

**Key Words :** sunflower, downy mildew, races.

# THE EFFECTS OF APPLIED HERBICIDES ON YIELD AND OIL QUALITY COMPONENTS OF TWO OLEIC AND TWO LINOLEIC SUNFLOWER (*Helianthus annuus* L.) HYBRIDS

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## ABSTRACT

The aim of the present study was to investigate the effect of application at the recommended dose by their manufacturer of five herbicides including different *active* ingredients herbicides on seed yields, some morphological and phenological characters, oil content of seed and oil fatty acid profiles of two oleic and two linoleic sunflower hybrids. One of oleic and linoleic hybrids were tolerant to the *imazamox* herbicide as the Clearfield trait. The experiments were conducted under field conditions during 2014 and 2015 in Lüleburgaz, Turkey. Used five traditional herbicides having different active ingredients as pre-plant, pre-emergence or post-emergence were Bonoflan WG with benfluralin, Stomp® Extra with pendimethalin, Challenge 600 with aclonifen, Targa Super with quizalofop-p-ethyl and Intervix® Pro with imazamox active ingredients. Intervix® Pro traditional herbicide with imazamox active ingredient decreased in plant heights of two Clearfield hybrids (LG 5542 CL and Colombi) about 10% in both years. This herbicide had insignificant negative effects on seed oil content and oleic aid ratio of Clearfield sunflower hybrid "LG 5542 CL" in 2014. Stomp® Extra with pendimenthalin active ingredient applied pre-emergence herbicide decreased the number of days to flowering of linoleic sunflower hybrid "64LL05" and oleic sunflower hybrid "64H34" in 2014 and 2015. In the other hand, Bonoflan WG with benfluralin application to "64H34" cultivar had the highest seed yield in 2015. Herbicide residue in harvested seeds of all applications was not detected upper than limits.

**Key words:** Fatty acids, herbicide application, sunflower, oil content, yield components

## INTRODUCTION

Weed control in crop production is very important. Crop yield decreases from 10% to 40% were observed when the sunflower was weeded during the first four weeks after emergence (Wanikorn, 1991; Delchev and Georgiev, 2015). The most common preferable method by the farmers for weed management is chemical application which is economical in short term and faster result to get rid of weeds. Agrochemicals are looked upon as a vehicle for improved crop production technology though it is a costly input. Pesticide use has become inevitable in modern agriculture, and increased several folds during the last four decades Over use of these chemicals have severe effects on environment that may lead to an immediate and long term effects (Sequtowski and Kortekamp, 2011; Bhandari, 2014)

There are so many study made on effects of pesticides on environment and human healthy. Degradation of these compounds in the environment and extensive or inappropriate use by farmers can lead to the contamination of various ecosystems. Widespread distribution of pesticides is also known to cause problems to the apiculture industry and in surface waters (Centner and Nicholas Eberhart, 2014; Lari et al., 2014). Severe effects of herbicides have also been announced in agency reports of International Organizations (EPA, 1996; FAO, 2013; EFSA, 2014).

Many pesticides are harmful, and cause to death for bee population. There are some pesticides that kill the bees directly. Since bees are the most important pollinators of crops, the use of pesticides can considerably reduce the yield of cross pollinated crops. Bees may be contaminated by pesticide residues during harvesting and contaminants can be transported on bee bodies or with forages to the hive, from where they can be transferred into honey (Ünal et al, 2010; Bargańska et al, 2014).

The persistence of pesticides in soil and their residual effects on sequential crops have been reported by many researchers (Wicks et al, 1969; Demircioğlu and Maden, 2007; Anonymous, 2009; Süzer and Büyük, 2010; Baranski et al, 2014: Serim and Maden, 2014). These chemicals (herbicides) are also caused some damage to crops or non-target plants due to wrong usage with technical implementation (Torun and Uygur, 2011).

The most of part of previous researches have been on the effects of the application of herbicides for weed control in weed-crop competition. Some of them were on crop productivity, and the grain yield and the oil content of sunflower seeds were also measured in order to confirm the importance of successful weed control (Simic et al., 2011; Reddy et. al., 2012; Knezevic et al., 2013.Petcu and Ciontu, 2014; Jursik et al., 2015; Suryavanshi et. al., 2015)

There were vanishingly small number research to determine the effects of herbicides at the normal dose on the yield and quality components of sunflower. The aim of the present study was to investigate the effect of application at the recommended dose by their manufacturer of five herbicides including different *active* ingredients on seed yields, some morphological and phenological characters, oil content of seed and oil fatty acid profiles of two oleic and two linoleic sunflower hybrids.

## MATERIALS AND METHODS

The data presented in this paper were collected as a part of a larger study to investigate the effect of application of five herbicides including different *active* ingredients on the some agronomic characters of sunflower in 2014 and 2015. Field experiments were conducted on farmer fields in Karamusul village (41º 24′ N, 27º 21′ E, elevation 46 m) of Lüleburgaz, Kırklareli at one of main sunflower-growing regions of Turkey. Some properties of experimental area soils were given in Table 1. Soil properties in both year were similar. The had clay loam texture. There was no any important problem in soil properties of experimental areas for sunflower production.

Table 1.Some chemical properties of the experimental field soil

| Year | SOM (%) | PH | Lime (%) | Salt (%) | N (%) | $P_2O_5$ ppm | K ppm | Ca ppm | Cu ppm | Fe ppm | Mn ppm | Mg ppm | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2014 | 1.92 | 6.7 | 0.57 | 0.07 | 0.08 | 14.28 | 212 | 4225 | 1.88 | 12.13 | 15.16 | 414 | 2.23 |
| 2015 | 1.88 | 6.8 | 0.68 | 0.08 | 0.09 | 14.36 | 228 | 4313 | 1.79 | 12.48 | 15.38 | 432 | 2.33 |

SOM = soil organic matter

Table 2 shows some meteorological data during two growth season. The second year was very dry after R6 growing period although rainfall was fairly well and steady during almost all vegetative and reproductive growth period in 2014.

Table 2.Climatic data during growing periods of Sunflower in 2014 and 2015

| Month | Rainfall (mm) | | Relative humidity (%) | | Temperature (°C) | |
|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| April | 47.0 | 69.8 | 83.6 | 75.3 | 12.5 | 11.1 |
| May | 80.0 | 5.8 | 79.9 | 69.5 | 16.9 | 18.8 |
| June | 51.4 | 42.8 | 76.2 | 69.2 | 21.2 | 21.3 |
| July | 131.6 | 4.8 | 73.4 | 65.3 | 23.8 | 24.5 |
| August | 19.2 | 2.6 | 73.8 | 63.1 | 24.2 | 25.3 |
| September | 121.4 | 63.0 | 81.8 | 74.2 | 18.9 | 21.8 |

Table 3 shows some properties of cultivars in this study. Two high oleic and two high linoleic sunflower hybrids were used. One of oleic and linoleic hybrids were tolerant to the *imazamox h*erbicide as the Clearfield technology.

Tablo 3. Some properties of sunflower cultivars in this study

| | Sunflower cultivar | Seed company | Clearfield/non-Clearfield | Oil fatty acid profile |
|---|---|---|---|---|
| 1 | LG 5542 CL | Limagrain | Clearfield | High Linoleic |
| 2 | 64LL05 | Pioneer | non-Clearfield | High Linoleic |
| 3 | Colombi | Syngenta | Clearfield | High Oleic |
| 4 | 64H34 | Pioneer | non-Clearfield | High Oleic |

Active ingredients, application rates, application times and trade names, manufacturer of herbicides.were given in Table 4. All herbicides were applied by backpack sprayer at the recommended dose from their manufacturer. “Intervix® Pro” was applied to Clearfield cultivars “LG 5542 CL and Colombi”. Other four herbicides “Bonaflan WG, Stomp® Extra, Challenge 600 and Targa Super” were applied to non-Clearfield cultivars “64LL05 and 64H34”. Each cultivar also had a control “untreated” plot for each replication.

Table 4. Active ingredients, application rates, application times and trade names, manufacturer of herbicides.

| | Trade name | Manufacturer | Active ingredient | Dose (ml/ha) | Application time |
|---|---|---|---|---|---|
| 1 | Bonaflan WG | Dow AgroSciences | Benfluralin, 60 g/ltr | 2500 | Pre-Plant |
| 2 | Stomp® Extra | BASF | Pendimethalin, 450 g/ltr | 3000 | Pre-Emergence |
| 3 | Challenge 600 | Bayer | Aclonifen, 600 g/ltr | 1250 | Post-Emergence |
| 4 | Targa Super | Sumi Agro | Quizalofop-P-Ethyl, 50 g/ltr | 1000 | Post-Emergence |
| 5 | Intervix® Pro | BASF | Imazamox, 40 g/ltr | 1250 | Post-Emergence |

The experiments were laid out in randomized complete block design (RCBD) with split plot arrangement having fourteen sub-plots including five different herbicide applications and untreated control plots on four sunflower cultivar with four replications in 2014 and 2015.

Each plot was set up in planting at 5.0 m × 2.8 m = 14.0 $m^2$. Planting was done on May 21, 2014 for the first year and on April 27, 2015 for the second year with an intra-row spacing of 30 cm and a row-to-row spacing of 70 cm. The reason of late planting in the first year was heavy rainfall. The experimental field in each year was fertilized as 300 kg $ha^{-1}$ with 20-20-0 (NPK) prior to sowing. In each growing season, observations such as plant height, time to flowering, head diameter, one thousand seeds weight, seed yield, oil and protein contents of seed, and oil fatty acids.

Pesticide (herbicide) residues analysis were done on harvested seeds from all plots belonging to herbicide applications within each block according to TS EN 15662 by private firm. In analyses, GC MS/MS and LC MS/MS instruments were used for benfluralin, and pendimethalin, aclonifen and imazamox, respectively. UPLC MS/MS instrument was used only to analysis of Quizalofop-P-Ethyl.

Statistical analysis was conducted according to Standard procedures for a randomized complete block design with split plot separately for Clearfield and non-Clearfield cultivars. The SAS System was used to generate the analysis of variance (ANOVA) for determining treatment effects on the dependent variables (SAS Institute, 1997). Treatment mean comparisons were based on F-Protected Least Significance Differences (LSD) comparisons at $P \leq 0.05$.

## RESULTS AND DISCUSSION

In this research, "Intervix® Pro" was applied to Clearfield cultivars "LG 5542 CL and Colombi". Other four herbicides "Bonaflan WG, Stomp® Extra, Challenge 600 and Targa Super" were applied to non-Clearfield cultivars "64LL05 and 64H34". Each cultivar had a control "untreated" plot within each replication. Thus, analysis of variance were done separately for Clearfield and non-Clearfield cultivars.

Table 5.Analysis of variance (mean square) of some sunflower yield and yield components with herbicide applications as separately for Clearfield and non-Clearfield cultivars in 2014 and 2015

| **Clearfield cultivars** | Plant height | Head diameter | Stem diameter | 1000 seed weight | Days to 50% flowering | Test weight | Seed yield |
|---|---|---|---|---|---|---|---|
| Year | 3333.97$^{**}$ | 4.22$^{**}$ | 0.29$^{ns}$ | 0.12$^{ns}$ | 11.28$^{*}$ | 0.01$^{ns}$ | 290.65$^{ns}$ |
| Cultivar (Cul) | 1275.0$^{**}$ | 18.06$^{**}$ | 2.31$^{**}$ | 25.10$^{ns}$ | 5.28$^{*}$ | 24.67$^{**}$ | 407.55$^{ns}$ |
| Application (App) | 1933.33$^{**}$ | 0.27$^{ns}$ | 4.33$^{**}$ | 45.13$^{ns}$ | 2.53$^{ns}$ | 0.34$^{ns}$ | 293.79$^{ns}$ |
| Year*Cul | 223.82$^{**}$ | 0.11$^{ns}$ | 0.49$^{*}$ | 0.17$^{ns}$ | 2.53$^{ns}$ | 0.03$^{ns}$ | 7378.08$^{*}$ |
| Year*App | 0.24$^{ns}$ | 0.45$^{ns}$ | 0.11$^{ns}$ | 0.25$^{ns}$ | 0.03$^{ns}$ | 9.14$^{ns}$ | 50.45$^{ns}$ |
| Cultivar*App | 12.09$^{*}$ | 1.74$^{ns}$ | 4.13$^{**}$ | 4.96$^{ns}$ | 1.53$^{ns}$ | 0.14$^{ns}$ | 13.49$^{ns}$ |
| Year*Cul*App | 16.06$^{*}$ | 1.24$^{ns}$ | 0.07$^{ns}$ | 0.01$^{ns}$ | 0.03$^{ns}$ | 0.02$^{ns}$ | 0.22$^{ns}$ |
| **C.V.** | **1.16** | **3.81** | **4.75** | **5.42** | **1.27** | **3.51** | **10.26** |
| **non-Clearfield cultivars** | | | | | | | |
| Year | 2472.87$^{**}$ | 48.88$^{**}$ | 1.62$^{*}$ | 0.16$^{ns}$ | 56.11$^{**}$ | 0.61$^{ns}$ | 63429.28$^{**}$ |
| Cultivar (Cul) | 416.42$^{**}$ | 177.40$^{**}$ | 0.74$^{*}$ | 164.37$^{*}$ | 66.61$^{**}$ | 1.46$^{ns}$ | 2921.07$^{ns}$ |
| Application (App) | 468.97$^{**}$ | 0.77$^{ns}$ | 1.18$^{**}$ | 51.67$^{ns}$ | 8.89$^{**}$ | 4.22$^{ns}$ | 1340.64$^{ns}$ |
| Year*Cul | 0.08$^{ns}$ | 2.96$^{ns}$ | 0.46$^{ns}$ | 0.77$^{ns}$ | 3.61$^{*}$ | 0.20$^{ns}$ | 3015.35$^{ns}$ |
| Year*App | 246.23$^{**}$ | 0.35$^{ns}$ | 0.09$^{ns}$ | 0.31$^{ns}$ | 0.46$^{ns}$ | 0.56$^{ns}$ | 563.62$^{ns}$ |
| Cultivar*App | 398.21$^{**}$ | 3.66$^{ns}$ | 1.92$^{**}$ | 193.17$^{**}$ | 9.27$^{**}$ | 3.18$^{ns}$ | 523.25$^{ns}$ |
| Year*Cul*App | 269.81$^{**}$ | 1.14$^{ns}$ | 0.33$^{ns}$ | 0.82$^{ns}$ | 1.33$^{*}$ | 0.55$^{ns}$ | 1052.91$^{ns}$ |
| **C.V.** | **2.11** | **9.83** | **7.17** | **7.22** | **1.00** | **3.81** | **14.37** |

* and ** : **: Significant differences based on ANOVA are shown at $P < 0.05$ and $P < 0.01$, respectively. ns: non significant

Tablo 5 shows analysis of variance of some sunflower yield and yield components with herbicide applications as separately for Clearfield and non-Clearfield cultivars in 2014 and 2015

According to ANOVA results, Herbicide application affected significantly at $P < 0.01$ on plant height, stem diameter of Clearfield cultivars. For the non-Clearfield cultivars, herbicide application had significant effect on plant height, stem diameter and days to 50% flowering.

Herbicide application to Clearfield cultivars had significant effect on stearic acid of seed oil while the effects of Year*Cultivar*Application interaction on oleic and linoleic acid of seed oil was significant at $P < 0.05$ statistical level (Table 6). For non-Clearfield cultivars, the effects

of Year*Cultivar*Application interaction on oil content and behenic acid (C22:0) were significant at $P < 0.01$ and $P < 0.05$, respectively.

Table 6.Analysis of variance (mean square) of oil content and fatty acid composition with herbicide applications as separately for Clearfield and non-Clearfield cultivars in 2014 and 2015

| **Clearfield cultivars** | Seed oil content | C16:0 Palmitic | C18:0 Stearic | C18:1 Oleic | C18:2 Linoleic | C22:0 Behenic | C24:0 Lignoceric |
|---|---|---|---|---|---|---|---|
| Year | 5.07 ns | 0.17 ns | 1.42** | 24.68 ns | 23.21 ns | 0.01 ns | 0.01 ns |
| Cultivar (Cul) | 30.69** | 14.20** | 1.22** | 13763.9** | 12667.2** | 0.01 ns | 0.01 ns |
| Application (App) | 0.99 ns | 0.03 ns | 0.06* | 1.04 ns | 1.66 ns | 0.01 ns | 0.01 ns |
| Year*Cul | 2.14 ns | 0.27* | 0.33** | 0.63 ns | 0.47 ns | 0.05 ns | 0.01 ns |
| Year*App | 1.62 ns | 0.12 ns | 0.01 ns | 13.55 ns | 7.73 ns | 0.02 ns | 0.01 ns |
| Cul*App | 0.12 ns | 0.14 ns | 0.03 ns | 0.13 ns | 0.34 ns | 0.01 ns | 0.01 ns |
| Year*Cul*App | 0.01 ns | 0.05 ns | 0.03 ns | 37.37* | 41.29* | 0.04 ns | 0.01 ns |
| **C.V.** | **3.39** | **4.61** | **3.45** | **4.69** | **10.18** | **19.34** | **23.77** |
| **non-Clearfield cultivars** | | | | | | | |
| Year | 257.22** | 0.01 ns | 0.01 ns | 449.07** | 654.25** | 0.19* | 0.01 ns |
| Cultivar (Cul) | 289.98** | 77.36** | 13.3 ** | 42187.6** | 38949.1** | 0.17* | 0.08 ns |
| Application (App) | 5.71 ns | 0.13 ns | 0.10 ns | 16.12 ns | 0.84 ns | 0.03 ns | 0.01 ns |
| Year*Cul | 61.65** | 0.57 ns | 0.37* | 19.52 ns | 5.09 ns | 0.04 ns | 0.03 ns |
| Year*App | 5.87 ns | 0.15 ns | 0.05 ns | 14.24 ns | 1.55 ns | 0.02 ns | 0.01 ns |
| Cul*App | 15.30* | 0.35 ns | 0.07 ns | 20.74 ns | 5.96 ns | 0.04 ns | 0.01 ns |
| Year*Cul*App | 13.93** | 0.38 ns | 0.04 ns | 19.01 ns | 7.08 ns | 0.07* | 0.01 ns |
| **C.V.** | **5.22** | **9.33** | **8.61** | **8.54** | **12.77** | **22.10** | **33.16** |

* and ** : **: Significant differences based on ANOVA are shown at $P < 0.05$ and $P < 0.01$, respectively. ns: non significant

Variations in yield and yield components by herbicide applications are given in Table 7. Intervix® Pro had negative effect on plant height. Plant height of Clearfield cultivars by Intervix® Pro with Imazamox (40 g/ltr) active ingredient was shortened 10.59% than untreated plots. This herbicide had also significant negative effect on stem diameter. The negative effects of Intervix® Pro on seed yield, test weight and head diameter of Clearfield cultivars were insignificant.

Although ANOVA of herbicide application shows insignificant effect on yield components of non-Clearfield cultivations, it created different $LSD_{0.05}$ groups for plant height, stem diameter, 1000 seed weight, days to 50% flowering, test weight and seed yield.

Targa Super with Quizalofop-P-Ethyl (50 g/ltr) application decreased seed yield per hectare according to other herbicides while it was in the same statistical group with untreated plots. Otherwise, Bonaflan WG application had the highest seed yield in the first group. The lowest plant height was measured in untreated plots. Oppositely to the effects of Intervix® Pro on Clearfield cultivars, other four herbicides application to non-Clearfield cultivars increased plant height according to untreated plots. Stomp® Extra with pendimenthalin active ingredient applied pre-emergence herbicide decreased the number of days to flowering of linoleic sunflower hybrid "64LL05" and oleic sunflower hybrid "64H34". It had positive effect on stem diameter and seed test weight.

Table 7. Variations in yield and yield components by herbicide applications

| | Plant height (cm) | Head diameter (cm) | Stem diameter (cm) | 1000 seed weight (g.) | Days to % 50 flowering | Test weight (kg/hl) | Seed yield (kg/ha) |
|---|---|---|---|---|---|---|---|
| **Clearfield cultivars** | | | | | | | |
| Untreated | 146.80 a | 18.61 | 6.20 a | 67.61 | 75.25 | 34.99 | 2437.98 |
| Intervix® Pro | 131.26 b | 18.42 | 5.47 b | 69.98 | 75.81 | 34.79 | 2377.38 |
| **$LSD_{0.05}$** | **1.19** | | **0.20** | | | | |
| | | **0.52** | | **2.74** | **0.71** | **0.90** | **181.68** |
| **non-Clearfield cultivars** | | | | | | | |
| Untreated | 113.96 c | 15.82 | 5.38 c | 73.79 ab | 72.06 a | 34.75 ab | 2036.5 ab |
| Bonaflan WG | 122.34 b | 15.49 | 5.54 bc | 76.25 a | 71.63 a | 34.89 a | 2251.3 a |
| Stomp® Extra | 126.43 a | 15.40 | 6.10 a | 71.78 b | 70.13 b | 35.25 a | 2147.6 ab |
| Challenge 600 | 122.25 b | 15.25 | 5.79 b | 74.94 ab | 71.69 a | 33.86 b | 2100.7 ab |
| Targa Super | 127.86 a | 15.64 | 5.69 b | 72.55 ab | 71.56 a | 34.74 ab | 2028.2 b |
| **$LSD_{0.05}$** | **1.83** | | **0.29** | **3.77** | **0.51** | **0.94** | **214.95** |
| | | **1.08** | | | | | |

*: Within each column for Clearfield and non-Clearfield cultivars, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.

Table 8 shows the variations in seed oil content and fatty acid compositions by herbicide applications. According to results, only stearic acid (C18:0) was affected by herbicide application. Intervix® Pro decreased content of stearic acid in seed oil of Clearfield cultivars.

Positive effects on seed oil content, linoleic acid and behenic acid, and negative effects on oleic acid and palmitic acid of this herbicide were insignificant at $P < 0.05$ statistical level. Insignificant positive effect of Bonaflan WG was observed on seed oil content. It also increased insignificantly palmitic and stearic acids. The highest oleic acid was found in untreated plots.

Table 8. Variations in seed oil content and fatty acid compositions by herbicide applications

| **Clearfield cultivars** | Seed oil content (%) | C16:0 Palmitic (%) | C18:0 Stearic (%) | C18:1 Oleic (%) | C18:2 Linoleic (%) | C22:0 Behenic (%) | C24:0 Lignoceric (%) |
|---|---|---|---|---|---|---|---|
| Untreated | 42.09 | 5.06 | 2.77 a | 61.76 | 28.62 | 0.78 | 0.34 |
| Intervix® Pro | 42.44 | 5.00 | 2.68 b | 61.40 | 29.08 | 0.81 | 0.35 |
| **LSD$_{0.05}$** | | | **0.07** | | | | |
| | **1.05** | **0.17** | | **2.13** | **2.16** | **0.11** | **0.06** |
| **non-Clearfield cultivars** | | | | | | | |
| Untreated | 42.31 | 4.58 | 2.90 | 63.21 | 27.61 | 0.74 | 0.31 |
| Bonaflan WG | 42.63 | 4.78 | 3.09 | 60.59 | 28.08 | 0.64 | 0.27 |
| Stomp® Extra | 42.32 | 4.61 | 3.06 | 62.47 | 28.12 | 0.72 | 0.28 |
| Challenge 600 | 41.24 | 4.65 | 3.00 | 62.72 | 27.87 | 0.72 | 0.30 |
| Targa Super | 42.76 | 4.55 | 2.95 | 62.61 | 28.16 | 0.75 | 0.33 |
| **LSD$_{0.05}$** | **1.56** | **0.31** | **0.18** | **3.77** | **2.53** | **0.11** | **0.07** |

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P< 0.05$.

Variations in some important yield and oil character according to cultivars, years and applications are given in Table 9. Plant height of LG 5542 CL and Colombi was affected negatively. by Intervix® Pro application in both years. In LG 5542 CL, Intervix® Pro application in 2014 and 2015 decreased plant height 12.28 and 11.44%, respectively. Decreases in Colombi were 7.93 and 11.09% for 2014 and 2015, respectively. Colombi in 2014 was affected negatively more than in 2015.

Table 9. Variations in some important yield and oil character according to cultivars, years and applications

| Cultivar | Year | Application | Seed yield (kg/ha) | Days to % 50 flowering | Plant height (cm) | Seed oil content (%) | C18:1 Oleic (%) | C18:2 Linoleic (%) |
|---|---|---|---|---|---|---|---|---|
| LG 5542 CL | 2014 | Untreated | 244.52 | 75.50 | 149.46a | 44.03 | 41.67 | 47.73 |
| | | Intervix® Pro | 254.22 | 76.50 | 131.10b | 43.78 | 37.98 | 51.23 |
| | | **LSD$_{0.05}$** | **45.13** | **3.18** | **7.00** | **2.37** | **9.49** | **8.83** |
| | 2015 | Untreated | 227.54 | 73.75b | 132.75a | 42.23 | 40.25 | 49.52 |
| | | Intervix® Pro | 222.52 | 74.75a | 117.56b | 42.95 | 43.48 | 46.51 |
| | | **LSD$_{0.05}$** | **27.41** | **0.99** | **2.24** | **4.23** | **4.62** | **4.44** |
| | 2014 | Untreated | 222.42 | 76.25 | 164.73a | 41.40 | 81.40 | 10.24 |
| | | Intervix® Pro | 229.86 | 76.25 | 151.66b | 41.46 | 81.77 | 9.62 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Colombi | | **LSD$_{0.05}$** | **40.54** | **1.30** | **1.30** | **3.51** | **8.26** | **8.99** |
| | | Untreated | 261.49 | 75.50 | 140.27a | 40.71 | 83.73 | 7.005 |
| | 2015 | Intervix® Pro | 263.58 | 75.75 | 124.71b | 41.59 | 82.38 | 8.96 |
| | | **LSD$_{0.05}$** | **29.02** | **0.80** | **2.35** | **3.16** | **2.33** | **2.80** |
| | | Untreated | 181.01 | 71.75 | 117.13d | 44.55b | 38.16 | 51.73 |
| | | Bonaflan WG | 178.31 | 71.50 | 122.00c | 44.45b | 36.46 | 52.91 |
| | 2014 | Stomp® Extra | 169.12 | 71.00 | 123.82c | 45.08ab | 34.58 | 54.94 |
| | | Challenge 600 | 168.36 | 71.50 | 128.46b | 45.21ab | 38.40 | 51.30 |
| 64LL05 | | Targa Super | 157.94 | 72.00 | 137.68a | 46.07a | 34.87 | 54.84 |
| | | **LSD$_{0.05}$** | **29.92** | **1.55** | **2.19** | **1.12** | **4.78** | **4.34** |
| | | Untreated | 230.38 | 69.75b | 115.10b | 42.70 | 42.57 | 46.68 |
| | | Bonaflan WG | 246.10 | 68.75c | 111.76c | 42.87 | 42.10 | 47.43 |
| | 2015 | Stomp® Extra | 246.10 | 68.75c | 111.76c | 43.50 | 42.01 | 46.69 |
| | | Challenge 600 | 233.80 | 68.75c | 110.89c | 44.34 | 41.84 | 47.07 |
| | | Targa Super | 241.32 | 71.25a | 124.28a | 42.79 | 42.58 | 46.72 |
| | | **LSD$_{0.05}$** | **45.14** | **0.69** | **2.36** | **3.43** | **3.30** | **3.66** |
| | | Untreated | 174.60 | 74.25a | 121.80c | 43.01ab | 83.23 | 8.61 |
| | | Bonaflan WG | 196.26 | 73.75a | 135.22b | 43.61a | 83.15 | 8.98 |
| | 2014 | Stomp® Extra | 210.89 | 71.00c | 135.32b | 42.88ab | 84.80 | 7.27 |
| | | Challenge 600 | 206.12 | 73.50ab | 139.28a | 43.00ab | 82.10 | 9.59 |
| 64H34 | | Targa Super | 188.69 | 72.25bc | 120.57d | 42.59b | 83.76 | 8.09 |
| | | **LSD$_{0.05}$** | **46.74** | **1.25** | **1.16** | **0.97** | **9.73** | **9.26** |
| | | Untreated | 228.61b | 72.50a | 101.80d | 38.98a | 88.87 | 3.41 |
| | | Bonaflan WG | 279.84a | 72.50a | 120.39b | 39.58a | 80.67 | 3.00 |
| | 2015 | Stomp® Extra | 232.94b | 69.75c | 134.83a | 37.82ab | 88.48 | 3.57 |
| | | Challenge 600 | 232.01b | 73.00a | 110.37c | 32.42b | 88.53 | 3.51 |
| | | Targa Super | 223.33b | 70.75b | 128.91a | 39.59a | 89.24 | 2.97 |
| | | **LSD$_{0.05}$** | **44.76** | **0.51** | **7.17** | **5.78** | **12.29** | **1.63** |

*: Within each column for each cultivar and year, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.

The second year depend on dry condition especially after R6 growth stage caused to stress on plants. It decreased seed oil contents. Thus, Intervix® Pro application in 2015 affected negatively some yield and quality components more than 2014. Generally, the decreases were insignificant. In seed yield and linoleic acid of LG 5542 CL by the herbicide application were also observed decreases in 2015 although oleic acid content of Colombi affected negatively in 2015. The otherwise, some insignificant increases by Intervix® Pro were determined similar in seed yield of LG 5542 CL in 2014 according to untreated plots.

In 64LL05 cultivar in 2014 and 2015 had the highest plant height by Targa Super application. Engrossingly, plant height of untreated plots was the lowest in 2014. Targa Super herbicide application also resulted the highest days number to flowering of 64LL05 in 2015. In addition, this herbicide application was in the first highest seed oil content group with Challenge

600 and Stomp® Extra in 2014. Although insignificant differences were found in seed yield of 64LL05 cultivar, untreated plots gave the highest seed yield in 2014. In the second year, Bonaflan WG and Stomp® Extra gave the highest seed yield in 64LL05.

In the other hand, Stomp® Extra and Targa Super decreased significantly days to the flowering and seed oil content of 64H34 in 2014. The other applications including untreated plots were in the latest group for flowering in this year. The highest plant height of 64H34 cultivar in 2014 was observed in Challenge 600 application while Targa Super application gave the lowest plant height. The differences among herbicide applications for oleic acid and linoleic acid content of 64H34 in 2014 were not significant at $P < 0.05$ statistical level. However, Stomp® Extra application had the highest seed yield and oleic content of seed oil. Untreated plots gave the lowest seed yield in 2014.

Herbicide application created statistically significant groups for seed yield, number of days to flowering, plant height and seed oil content of 64H34 in 2015. Bonaflan WG application to 64H34 in 2015 gave the highest seed yield. It increased significantly seed yield according to untreated plots and other herbicide applications. Although Targa Super was in the second group with the other applications except Bonaflan WG, it created the lowest seed yield. Stomp® Extra decreased the number of days to flowering 64H34 in 2015 similar to the first year. The highest plant height was also measured in Stomp® Extra with Targa Super application although Targa Super had negative effect on plant height in 2014.

Results of pesticide (herbicide) residues analysis on harvested seeds belonging to herbicide applications are given in Table 10. Analyses were done according to TS EN 15662. In analyses, GC MS/MS and LC MS/MS instruments were used for benfluralin, and pendimethalin, aclonifen and imazamox, respectively. UPLC MS/MS instrument was used only to analysis of Quizalofop-P-Ethyl. Active ingredient residue on harvested seed from herbicide applications belonging to each block and each replication were not detected according to limit.

Table 10. Pesticide (Herbicide) residues analysis on harvested seeds

| Trade name | Active ingredient | Limit (LOQ) | Unit | Result | Instrument Analysis method |
|---|---|---|---|---|---|
| Bonaflan WG | Benfluralin, 60 g/ltr | 0.01 | mg/kg | Not Detected | GC MS/MS TS EN 15662 |
| Stomp® Extra | Pendimethalin, 450 g/ltr | 0.01 | mg/kg | Not Detected | LC MS/MS TS EN 15662 |
| Challenge 600 | Aclonifen, 600 g/ltr | 0.01 | mg/kg | Not Detected | LC MS/MS TS EN 15662 |
| Targa Super | Quizalofop-P-Ethyl, 50 g/ltr | 0.01 | mg/kg | Not Detected | UPLC MS/MS J.of AOAC Int. Vol. 90. No.2.2017 |
| Intervix® Pro | Imazamox, 40 g/lt | 0.01 | mg/kg | Not Detected | LC MS/MS TS EN 15662 |

Delchev and Georgiev (2015) and Suryavanshi et al. (2015) also reported results in the same direction with this study. But they usually emphasized the effective and efficient use of pesticides. Agrochemicals (pesticides and fertilizers) are looked upon as a vehicle for improved crop production technology though it is a costly input. Balance use, optimum doses, correct

method and right time of application of agrochemicals ensures increased crop production. The requirement of fertilizers and pesticides for crops differ according to soil and meteorology. On a large scale, success of pesticide application is depend on farmer knowledge and education (Bhandari, 2014).

The results show that some of herbicides could have hormonal positive effect on some characters with yield, morphological, physiological, seed oil content and fatty acid composition of sunflower while the effects of others are negative or in significant. It is a great result we could not find residue of herbicides in harvested seeds from application plots. The results lead to need more new researches on determination stress or hormonal effects of pesticides under different ecological conditions for evaluating effects of genotype, growth stage and environmental.

## ACKNOWLEDGEMENT

This article was submitted from the BAP project supported by Namık Kemal University, Turkey with NKUBAP.00.24.AR.14.29 project number as the title "The dermination of herbicides on yield and quality components, and residues in seed of sunflower (*Helianthus annuus* L.).

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# GENETIC CHARACTERIZATION OF THE INTERACTION BETWEEN SUNFLOWER AND OROBANCHE CUMANA.

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## ABSTRACT

*Orobanche cumana* is a major disease in cultivated areas around the black sea and in Spain. The pathogen spread recently to several other countries (France, China …). During the last ten years, several new *O. cumana*races have emerged but very few efficient methods were available to control their development. Genetic resistance was the more efficient and introgression of major resistance loci was successfully used to produce new resistant sunflower varieties. With the recent emergence of new virulent races, novel resistance loci need to be mapped and characterized. A recombinant inbred line population, derived from the cross between the lines HA89 and LR1, was used to map QTLs controlling quantitative resistance to race F. The phenotyping has been conducted on the 107 lines of the population at different stages of the interaction. We evaluated each line for (i) the capacity of their root exudate to induce germination of *O. cumana* seeds, (ii) their ability to induce incompatible attachment, (iii) the number of broomrape tubercles in growth chamber, and (iv) the number of broomrape emergences in the field. Different response profiles were observed at these 4 stages of development, indicating several resistance mechanisms in sunflower. Interestingly, even if the two parental lines showed a close resistant phenotype, we observed a large diversity of the resistance level in the population. Combined with this detailed phenotyping analysis, we performed the genotyping of the sunflower recombinant inbred lines using an AXIOM array of 586 985 SNPs. QTLs will be mapped for the different traits.

**Key Words :** Orobanche cumana, sunflower, QTL, resistance

# ISOLATION AND IDENTIFICATION OF PATHOGEN OF SUNFLOWER *FUSARIUM* WILT

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## ABSTRACT

Fusarium spp. is one of the most destructive fungi which could infect a wide range of plants and cause wilt diseases. After infection, the lower leaves of sunflower showed the typical symptoms as discoloration, irregular, mottled and wilt. In the field, the symptom of the Fusarium wilt is very similar with sunflower yellow wilt caused by *Verticillium dahliae*. It is rather difficult to distinguish them. In August of 2014-2015, sunflower wilt samples, showing the above described symptom, were collected from Inner Mongolia, Jilin, Liaoning, Nixia and Xinjiang province of China. The pathogen was isolated from the diseased stem and identified the pathogen with Koch's postulates. The CLA, SNA and PDA medium were used to observe three types of conidias such as macroconidia, microconidia, and chlamydospores. Combined the morphology characteristic and the PCR results (with ITS and EF-1α primes), 31 isolates were identified as seven different species of *Fusarium* spp., including *F. oxysporum* (9 strains), *F. verticilloides* (2 strains), *F. lateritium* (1 strain), *F.acuminatum* (4 strains), *F. redolens* (2 strains), *F. equiseti* (2 strains) and *F. proliferum* (11 strains). Inoculation test was performed with both the stem or root wound inoculation method. All 31 strains could cause the wilt of sunflower seedling under lab condition. In conclusion, 7 species of Fusarium spp. was the causal agent of sunflower fusarium wilt in China and *F. proliferum* and *F. oxysporum* are the dominant species of sunflower fusarium wilt.

**Key Words :** Sunflower wilt; Koch's postulates ; Fusarium species.

# PCR COMBINED WITH GFP TAGGED VERTICILLIUM DAHLIAE CONFIRMED THE SEEDS TRANSMISSION OF SUNFLOWER *VERTICILLIUM* WILT

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## ABSTRACT

*Verticillium* wilt of sunflower (*Helianthus annuus* L.) is a widespread and destructive disease caused by the soil-borne fungal pathogen *Verticillium dahliae* (*V. dahliae*). The quick spreading of Verticillium Wilt in sunflower planting region of China promoted us to consider the possibility of seeds transmission the pathogen. Therefore, knowledge on the contamination of the seeds by *V. dahliae* is critical for understanding the infection cycle of sunflower yellow wilt and also to develop the efficient ways to control the spreading of this disease. In this study, sunflower seedlings were inoculated with conidial suspensions of GFP tagged isolate. Colonization and developing were studied under confocal microscopes. After 12 to 96 hour post-inoculation (hpi), conidia germinated and formed hyphal colonies on the root tips and in the root elongation zones. Hyphae colonized in cortical tissues and vascular elements after 2 weeks inoculation (2wpi). 10 wpi later, the xylem of the upper stem, sunflower disc including the pericarp and seed coat, had been colonized by the pathogen. Moreover, pathogen DNA could also be detected by PCR in the pericarp and seed coat. Additional experiment was performed to detect the transmission rate of seeds of different sunflower cultivars was conducted with PCR. Our result indicated that the transmission rate of sunflower seeds ranged from 10-25% among all tested cultivars. In conclusion, seed transmission is the main way for the long distance transmission of sunflower *V. dahliae* and seed pretreatment should be done to control the infection of sunflower seedling in the future.

**Key Words :** sunflower (*Helianthus annuus* L.); *Verticillium dahliae* ; seed transmission

# RAPID INVITRO SCREENING OF SUNFLOWER GENOTYPES FOR MOISTURE STRESS TOLERANCE USING PEG-6000

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## ABSTRACT

Sunflower is an important oilseed crop, cultivated mainly under rainfed situation the productivity is very low and experiences moisture stress during flowering and terminal growth stages. To overcome such situations, there is need to identify the moisture stress tolerant genotypes to develop drought tolerant varieties and hybrids. Present experiment was conducted mainly to screen large number of genotypes for moisture stress tolerance at laboratory level using chemical PEG-6000 (Poly Ethyl Glycol) at MARS, UAS, Raichur. A total of 160 genotypes screened with two replications of each treatment (0, 10, 15, 20, 25 and 30% of PEG-6000) in two factorial CRD fashion. All the treatments, genotypes and G X T interactions were significant for all five characters studied. The treatment mean indicated the germination per cent, shoot length, seedling length and seed vigour reduced drastically with increased concentrations of PEG. Based on the drastic reduction in seed germination and growth at two critical concentrations 20% and 25% PEG were considered for screening the germplasm. The genotype x treatment interactions at 20 and 25% PEG exhibited significant differences for few genotypes studied, hence these concentrations are considered as critical doses to identify/isolate the real moisture tolerant genotypes. Comparative performance indicated that shoot length was most affected one among the characters studied. From this study the moisture stress tolerant B-lines, R-lines and germplasm lines are identified as most tolerant lines to moisture stress induced by PEG-6000 and further suggesting that these inbred lines could be used as parental lines to develop moisture stress tolerant hybrids and varieties in the heterosis breeding programme

**Key words:** PEG-6000, Germination per cent, Root length

## INTRODUCTION

Sunflower (*Helianthus annuus* L.) plant belongs to *Asteracae* or *Compositae* family and it is native to the temperate North America. It is a highly cross pollinated crop which is adaptable to a wide range of agro climatic situations, having high yield potential, suitable for cultivation in all seasons due to its day neutral nature (photo insensitivity) and can fit well in various inter and sequence cropping systems. In India, the major area is under rainfed cultivation. The crop experiences severe moisture stress during flowering and terminal growth stages. In order to overcome moisture stress at such critical stages of crop, there is need identify the moisture stress tolerant genotypes at germination stage itself. Germination stage is an important stage to maintain adequate population for obtaining prominent yield. Hence the present investigation was mainly to identify genotypes which can grow under moisture stress conditions through germination test under laboratory conditions using moisture stress inducing agent PEG-6000 having a molecular weight of 6000. Geetha *et al.,* (2012) proposed the use of PEG-6000 for

screening for drought tolerance under laboratory conditions and may be useful complementary method to field screening to overcome difficulties like uncontrolled climatic conditions, heterogeneity of soil, large amount of plant material to and time and labour consumption making field trials difficult for drought screening of genotypes. Turhan and Baser (2004) suggested that prior to field trial; an *in vitro* approach could be useful in screening and selecting for drought response. Ahmad *et al.* (2009) identified G-101 and 64-A-93 from *in vitro* response of sunflower genotypes to drought stress imposed at germination and seedling growth stages at five water stress levels induced by PEG.

## MATERıALS AND METHODS

*In vitro* screening of genotypes is an alternate and easy method to screen large number of genotypes with limited space and time, a total of 160 genotypes which includes (12 maintainer lines, 12 restorer lines and 136 germplasm lines) were screened by using a water stress inducing chemical i.e. poly ethylene glycol (PEG-6000). Many studies indicated that PEG-6000 is water stress inducing agent and it is best to screen germplasm for drought stress tolerance under laboratory conditions (Turhan and Baser, 2004; Somers *et al.* 1983; Ahmad *et al.* 2009 and Geetha *et al.* 2012).

Totally there are six treatments (0 control) 10, 15, 20, 25 and 30 g of weighed powder of PEG-6000 are dissolved in 100 ml of distilled water separately to prepare 0 (control), -0.6 (10 %), -0.9 (15 %), -1.2 (20 %), -1.5 (25 %) and -1.8 (30 %) M Pa concentrations, respectively and two replications were maintained for each osmotic potential in two factorial-CRD fashion. Twenty seeds of each genotype were rinsed and soaked for ten minutes using distilled water, later kept the seeds on the germinating paper in each petri plate and then treated with different concentrations of PEG-6000 and observations were recorded on germination per cent, root length, shoot length, seedling length and seed vigour seven days after sowing in petriplates.

The germination per cent, seedling length and seed vigour was calculated by using the formulae.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

Seedling length (cm) = Total length of shoot (cm) + Total length of root (cm).

Seed vigour= [Seedling length (cm) × Germination percentage]

## RESULTS AND DISCUSSION:

The analysis of variance for five characters on 160 sunflower genotypes under laboratory screening using PEG-6000 is presented in the Table 1. The analysis of variance exhibited that all the treatments, genotypes and genotypes x treatment interactions are found to be highly significant for all the five characters studied.

The mean treatment effects obtained for five characters are presented in Table 2. The significant differences were noticed for all the characters at (0 control), 10 and 15 per cent concentrations of PEG-6000. But 20, 25 and 30 per cent of PEG-6000 concentration none of the character found significant except germination percent. The overall treatment means indicated that germination per cent, shoot length and seedling length are drastically reduced with increasing the concentrations of PEG-6000 (20, 25 & 30%). But indicating that 20% and 25% concentration are the critical doses for screening moisture stress tolerant lines.

The results of the genotype x treatment interaction of 160 sunflower genotypes for five characters under laboratory evaluation are presented in Table 3. The *per se* performance of interaction effects of 160 sunflower genotypes are screened by using PEG-6000 at 0, 10, 15, 20, 25 and 30 per cent concentrations. The results revealed that at 10 and 15 per cent PEG majority of the genotypes showed highly significant mean *per se* performance. At 30 per cent PEG-6000 majority of the genotypes could not survive and could not exhibit significant *per se* performance for the characters studied and 30 per cent PEG acted as lethal dose. At 20 percent PEG, the germination percent ranged from 12.50 to 100 while at 25 per cent PEG it ranged from 5.0 to 95 percent. The root length ranged from 0.52 to 12.65 cm and 0.10 to 6.56 cm at 20 and 25 per cent PEG respectively. Shoot length ranged from 0.25 to 2.50cm and 0.12 to 1.20cm at 20 and 25 per cent PEG respectively. Similarly the seedling length ranged from 0.80 to 14.09 and 0.30 to 7.76cm at 20 and 25 per cent PEG respectively. Hence, the genotypic treatment interaction at 20 and 25 per cent were chosen to compare with control.

The germination per cent showed highly significant differences for majority genotypes at 0 per cent (control). The germination per cent at 20 per cent indicated 94 out of 160 genotypes were showed significant *per se* performance, while, at 25 per cent PEG, 24 out of 160 genotypes were found to be significant in comparison with control. Among CMS lines CMS 857B, among restorer lines R-78, R-12-2 and among germplasm lines $GP_6$-305, $GP_6$-2255, $GP_6$-371, $GP_6$-424, $GP_6$-442, $GP_6$-863, $GP_6$-11, $GP_6$-969, $GP_6$-310, $GP_6$-118, $GP_6$-211, $GP_6$-1576, $GP_6$-326, $GP_6$-714, $GP_6$-967, $GP_6$-1060, $GP_6$-366, $GP_6$-614, $GP_6$-54, $GP_6$-1102 and $GP_6$-1072 exhibited significant *per se* performance. The majority of the genotypes showed decreased the germination percent with increased concentrations of PEG-6000 compared to control. These results accordance with reports of Kaya *et al.* (2006), Iqbal and Asraf (2006), El Midaoui (2003), Ahmad *et al.* (2009) and Saensee *et al.* (2012). However at 15 per cent or its equivalent M pa reduced germination per cent was reported by Geetha *et al.* (2012) and Sheidaie *et al.* (2012).

The root length at 20 per cent PEG indicated 62 out of 160 genotypes were showed significant. While, at 25 per cent concentration five genotypes were found to be significant in comparison with control. Among CMS lines CMS 857B, among restorer lines R-78 and among germplasm lines GP6-1072, GP6-714 and GP6-1254 exhibited significant *per se* performance. The majority of the genotypes exhibited decreased root length at higher concentrations (25 and 30 % PEG) compared to control these results accordance with reports of Kaya *et al.* (2006), however some authors El. Midaoui *et al.* (2003), Geetha *et al.* (2012) and Sheidaie *et al.* (2012) reported restricted root length at 15 per cent PEG-6000 or its equivalent M pa.

The shoot length at 20 per cent concentration indicated only two out of 160 genotypes showed significant *per se* performance, while at 25 per cent concentration none of the genotypes were found to be significant. At higher concentrations of PEG majority of the genotypes showed decreased shoot length compared to control. These results are in accordance with reports of Kaya *et al.* (2006) and Ahmad *et al.* (2009). However some of the authors El Midaoui *et al.* (2003), Geetha *et al.* (2012) and Sheidae *et al.* (2012) reported that drastic reduction in shoot length drastically reduced at 15 per cent of PEG-6000. Fulda *et al.* (2010) reported the shoot growth was more affected than root growth at more than10 per cent of PEG-6000.

The seedling length at 20 per cent PEG indicated 33 out of 160 genotypes showed significant *per se* performance, while, at 25 per cent PEG only one genotype R-78 exhibited significant *per se* performance. The seedling length was arrested at higher concentrations of PEG-6000. These results are in accordance with the reports of Khodarampour (2011) in maize at

20 per cent PEG or its equivalent Mpa. Manuhara *et al.* (2013) reported sunflower callus growth inhibited with increased concentration of PEG-6000.

Seed vigour at 20 per cent PEG indicated 26 out of 160 genotypes showed significant *per se* performance, while at 25 per cent PEG none of the genotypes were found to be significant. The seed vigour decreased with increased concentration of PEG-6000, these results are in accordance with the reports of Barros and Rosseto (2009).

Comparative performance of 160 sunflower genotypes under laboratory conditions using water stress inducing chemical PEG-6000 at 20 and 25 per cent concentrations are presented in Table 4 for five characters. The germination per cent under 20 and 25 per cent concentrations reduced by -25.06 and -58.14 per cent, respectively compared to control (0 %), the root length was also reduced by -25.88 to -68.81 %, the shoot length was reduced by -86.30 to -93.53 %, seedling length was reduced from -56.76 to -81.45 %, seed vigour reduced by -65.14 to -89.50 %. Based on overall mean performance 38 promising genotypes were identified *viz.*, CMS 857B, CMS 104B, CMS 378B, R 78, R-12-2, R 630, GM 71R, $GP_6$-305, $GP_6$-371, $GP_6$-118, $GP_6$-714, $GP_6$-1072, $GP_6$-325, $GP_6$-2255, $GP_6$-424, $GP_6$-442, $GP_6$-863, $GP_6$-11, $GP_6$-969, $GP_6$-310, $GP_6$-211, $GP_6$-1576, $GP_6$-326, $GP_6$-967, $GP_6$-1060, $GP_6$-366, $GP_6$-614, $GP_6$-54, $GP_6$-1102, $GP_6$-1254, $GP_6$-1616, $GP_6$-420, $GP_6$-219, $GP_6$-517, $GP_6$-912, $GP_6$-734, $GP_6$-586 and $GP_6$-589 found to be moisture tolerant genotypes.

**Table 1. Analysis of variance for five different characters in sunflower under laboratory evaluation using PEG-6000**

| Source | DF | Germination per cent | Root length (cm) | Shoot length (cm) | Seedling length (cm) | Seed vigour |
|---|---|---|---|---|---|---|
| Treatment | 5 | 384834.50** | 3121.93** | 1838.55** | 7956.16** | 80230000.00** |
| Genotype | 159 | 1853.89** | 30.13** | 4.343** | 48.714** | 474957.23** |
| Treatment x Genotype | 795 | 344.26** | 5.76** | 1.62** | 9.50** | 97651.51** |
| Error | 960 | 13.25 | 0.06 | 0.03 | 0.11 | 1740.33 |

**Table 2. Treatment means of six different concentrations of PEG-6000 for five characters in sunflower**

| Treatment | Germination per cent | Root length (cm) | Shoot length (cm) | Seedling length (cm) | Seed vigour |
|---|---|---|---|---|---|
| 0 | 96.35** | 6.22** | 6.50** | 12.72** | 1228.00** |
| 10 % | 91.84** | 8.41** | 3.22** | 11.63** | 1074.00** |
| 15 % | 86.50** | 7.24** | 1.85 | 9.08** | 797.99** |
| 20 % | 72.20** | 4.61 | 0.89 | 5.50 | 428.06** |
| 25 % | 40.33 | 1.94 | 0.42 | 2.36 | 128.63 |
| 30 % | 8.47 | 0.37 | 0.13 | 0.50 | 11.60 |
| Mean | 65.95 | 4.80 | 2.17 | 6.96 | 228.10 |
| S E | 0.20 | 0.01 | 0.01 | 0.02 | 2.33 |
| CD 5% | 0.56 | 0.04 | 0.03 | 0.05 | 6.46 |
| CD 1% | 0.74 | 0.05 | 0.04 | 0.07 | 8.51 |

Table 3: Genotype x Treatment interaction effects for five different characters using PEG 6000 at 0, 20 and 25 % concentrations

| Sl. No | Genotypes | Germination per cent | | | Root length (cm) | | | Shoot length (cm) | | | Seedling length (cm) | | | Seed vigour | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PEG 6000 concentrations | | | PEG 6000 concentrations | | | PEG 6000 concentrations | | | PEG 6000 concentrations | | | PEG 6000 concentrations | | |
| | | 0 % | 20 % | 25 % | 0 % | 20 % | 25 % | 0 % | 20 % | 25 % | 0 % | 20 % | 25 % | 0 % | 20 % | 25 % |
| 1 | CMS-17B | 72.50 | 35.00 | 0.00 | 3.02 | 1.70 | 0.00 | 3.04** | 0.43 | 0.00 | 6.06 | 2.13 | 0.00 | 440.05 | 75.50 | 0.00 |
| 2 | CMS-103B | 90.00** | 50.00 | 35.00 | 3.50 | 2.67 | 1.95 | 3.52** | 0.90 | 0.65 | 7.02 | 3.57 | 2.60 | 631.80 | 179.10 | 91.00 |
| 3 | CMS-104B | 97.50** | 72.50 | 32.50 | 2.00 | 5.92** | 2.80 | 2.84** | 0.74 | 0.37 | 4.84 | 6.66 | 3.17 | 471.15 | 482.40 | 103.20 |
| 4 | CMS-148B | 100.00** | 100.00** | 70.00 | 1.60 | 2.81 | 0.95 | 3.50** | 0.59 | 0.33 | 5.10 | 3.40 | 1.28 | 510.00 | 340.00 | 89.60 |
| 5 | CMS-335B | 100.00** | 90.00** | 65.00 | 3.47 | 2.11 | 1.53 | 3.70** | 0.62 | 0.35 | 7.17 | 2.73 | 1.88 | 717.00* | 245.70 | 122.20 |
| 6 | CMS-338B | 100.00** | 90.00** | 45.00 | 3.37 | 1.91 | 0.68 | 3.80** | 1.00 | 0.35 | 7.17 | 2.91 | 1.03 | 717.00* | 262.45 | 46.50 |
| 7 | CMS-351B | 100.00** | 85.00** | 40.00 | 5.31* | 3.85 | 0.12 | 3.00** | 0.62 | 0.20 | 8.31** | 4.47 | 0.32 | 831.00** | 379.20 | 12.60 |
| 8 | CMS-378B | 100.00** | 75.00* | 45.00 | 3.80 | 3.70 | 1.39 | 5.96** | 0.54 | 0.22 | 9.76** | 4.24 | 1.61 | 976.00** | 318.50 | 71.60 |
| 9 | CMS-607B | 92.50** | 65.00 | 0.00 | 2.70 | 0.76 | 0.00 | 2.70** | 0.41 | 0.00 | 5.40 | 1.17 | 0.00 | 498.75 | 77.00 | 0.00 |
| 10 | CMS-850B | 100.00** | 95.00** | 45.00 | 3.90 | 2.12 | 0.70 | 4.90** | 0.82 | 0.28 | 8.80** | 2.94 | 0.98 | 880.00** | 280.00 | 43.88 |
| 11 | CMS-852B | 100.00** | 95.00** | 65.00 | 5.10 | 2.52 | 0.73 | 5.18** | 0.75 | 0.32 | 10.28** | 3.27 | 1.05 | 1030.00** | 310.65 | 68.70 |
| 12 | CMS-857B | 100.00** | 95.00** | 90.00** | 1.48 | 7.71** | 6.16** | 3.40** | 0.68 | 0.54 | 4.88 | 8.39** | 6.70 | 488.00 | 797.20** | 603.00 |
| 13 | R-12-2 | 100.00** | 100.00** | 80.00** | 5.22 | 6.68** | 2.91 | 4.50** | 1.69 | 0.63 | 9.72** | 8.37** | 3.54 | 972.00** | 837.00** | 283.20 |
| 14 | R 127-1 | 92.50** | 87.50** | 27.50 | 1.61 | 2.56 | 1.11 | 8.10** | 1.36 | 0.30 | 9.71** | 3.92 | 1.41 | 897.70** | 342.80 | 39.03 |
| 15 | R 64NB | 100.00** | 82.50** | 60.00 | 3.71 | 3.56 | 1.49 | 3.76** | 0.86 | 0.42 | 7.47 | 4.42 | 1.91 | 747.00** | 364.90 | 114.55 |
| 16 | R-78 | 100.00** | 95.00** | 87.50** | 8.20** | 7.85** | 6.56** | 7.10** | 1.54 | 1.20 | 15.30** | 9.39** | 7.76** | 1530.00** | 891.90** | 678.95 |
| 17 | R-630 | 100.00** | 87.50** | 37.50 | 5.46** | 4.95 | 2.94 | 4.74** | 0.99 | 0.51 | 10.20** | 5.94 | 3.45 | 1020.00** | 519.20 | 129.25 |
| 18 | GM-27R | 77.50** | 60.00 | 37.50 | 6.48** | 2.35 | 1.65 | 5.96** | 1.40 | 0.99 | 12.44** | 3.75 | 2.64 | 963.85** | 225.00 | 99.00 |
| 19 | GM-37R | 92.50** | 75.00* | 7.50 | 3.42 | 1.58 | 0.90 | 3.00** | 0.83 | 0.40 | 6.42 | 2.41 | 1.30 | 593.10 | 179.95 | 9.50 |
| 20 | GM-41R | 100.00** | 95.00** | 50.00 | 3.22 | 2.62 | 1.89 | 3.80** | 0.91 | 0.43 | 7.02 | 3.53 | 2.32 | 702.00* | 336.20 | 116.00 |
| 21 | GM-44R | 82.50** | 57.50 | 55.00 | 3.10 | 2.41 | 1.97 | 3.64** | 0.94 | 0.66 | 6.74 | 3.35 | 2.63 | 555.55 | 193.00 | 145.00 |
| 22 | GM-56R | 90.00** | 55.00 | 25.00 | 7.52** | 4.88 | 0.30 | 3.72** | 1.51 | 0.40 | 11.24** | 6.39 | 0.70 | 1010.00** | 352.00 | 18.50 |
| 23 | GM-59R | 90.00** | 50.00 | 30.00 | 5.20 | 2.41 | 1.80 | 3.76** | 1.40 | 0.54 | 8.96** | 3.81 | 2.34 | 806.40** | 191.05 | 70.20 |
| 24 | GM-71R | 90.00** | 70.00 | 60.00 | 7.76** | 3.97 | 2.90 | 5.36** | 0.88 | 0.24 | 13.12** | 4.85 | 3.14 | 1180.00** | 339.50 | 188.40 |
| 25 | $GP_6$-11 | 100.00** | 90.00** | 90.00** | 6.68** | 3.56 | 2.35 | 6.74** | 1.03 | 0.75 | 13.42** | 4.59 | 3.10 | 1340.00** | 413.10 | 279.00 |
| 26 | $GP_6$-18 | 100.00** | 85.00** | 15.00 | 6.04** | 3.20 | 1.80 | 5.96** | 0.71 | 0.38 | 12.00** | 3.91 | 2.18 | 1200.00** | 332.50 | 34.10 |
| 27 | $GP_6$-18-1 | 95.00** | 90.00** | 20.00 | 8.03** | 1.99 | 0.93 | 9.50** | 0.60 | 0.46 | 17.53** | 2.59 | 1.39 | 1670.00** | 233.10 | 27.80 |
| 28 | $GP_6$-54 | 100.00** | 87.50** | 75.00* | 7.51** | 6.87** | 2.61 | 5.44** | 0.80 | 0.57 | 12.95** | 7.67* | 3.18 | 1300.00** | 670.60 | 238.50 |
| 29 | $GP_6$-63 | 95.00** | 60.00 | 22.50 | 6.04** | 3.87 | 1.17 | 6.90** | 0.54 | 0.33 | 12.94** | 4.41 | 1.50 | 1230.00** | 264.25 | 34.25 |
| 30 | $GP_6$-83 | 95.00** | 77.50** | 27.50 | 5.22 | 5.42* | 2.70 | 6.84** | 0.87 | 0.82 | 12.06** | 6.20 | 3.52 | 1150.00** | 488.25 | 96.65 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | $GP_6$-109 | 100.00** | 60.00 | 10.00 | 6.27** | 2.26 | 0.45 | 8.04** | 0.55 | 0.30 | 14.31** | 2.81 | 0.75 | 1430.00** | 168.60 | 7.50 |
| 32 | $GP_6$-118 | 100.00** | 90.00** | 85.00** | 10.16** | 5.44** | 3.88 | 7.18** | 0.98 | 0.32 | 17.34** | 6.42 | 4.20 | 1730.00** | 577.80 | 357.00 |
| 33 | $GP_6$-127 | 95.00** | 72.50 | 30.00 | 5.34* | 3.90 | 0.82 | 6.28** | 1.74 | 0.45 | 11.62** | 5.64 | 1.27 | 1100.00** | 409.45 | 37.45 |
| 34 | $GP_6$-135 | 95.00** | 55.00 | 15.00 | 5.42* | 3.04 | 0.85 | 4.60** | 0.68 | 0.26 | 10.02** | 3.72 | 1.11 | 951.90** | 205.90 | 16.65 |
| 35 | $GP_6$-139 | 90.00** | 42.50 | 22.50 | 5.45** | 4.13 | 1.19 | 6.70** | 0.32 | 0.12 | 12.15** | 4.45 | 1.31 | 1090.00** | 188.80 | 29.75 |
| 36 | $GP_6$-160 | 95.00** | 40.00 | 0.00 | 3.05 | 1.30 | 0.00 | 5.40** | 0.80 | 0.00 | 8.45** | 2.10 | 0.00 | 802.75** | 84.00 | 0.00 |
| 37 | $GP_6$-173 | 100.00** | 82.50** | 65.00 | 7.71** | 5.54** | 2.05 | 6.24** | 1.01 | 0.51 | 13.95** | 6.55 | 2.56 | 1400.00** | 539.75 | 166.40 |
| 38 | $GP_6$-175 | 100.00** | 67.50 | 22.50 | 4.62 | 1.88 | 2.34 | 4.86** | 0.53 | 0.39 | 9.48** | 2.41 | 2.73 | 948.00** | 162.60 | 61.75 |
| 39 | $GP_6$-176 | 82.50** | 57.50 | 47.50 | 4.80 | 4.62 | 3.84 | 7.90** | 1.10 | 0.59 | 12.70** | 5.72 | 4.43 | 1050.00** | 328.50 | 210.90 |
| 40 | $GP_6$-181 | 100.00** | 50.00 | 0.00 | 4.82 | 4.99 | 0.00 | 4.92** | 0.25 | 0.00 | 9.74** | 5.24 | 0.00 | 974.00** | 262.40 | 0.00 |
| 41 | $GP_6$-211 | 100.00** | 100.00** | 85.00** | 7.72** | 7.27** | 3.43 | 7.10** | 0.95 | 0.53 | 14.82** | 8.22** | 3.96 | 1480.00** | 822.00** | 336.60 |
| 42 | $GP_6$-217 | 100.00** | 90.00** | 7.50 | 7.86** | 7.11** | 0.90 | 5.26** | 0.39 | 0.35 | 13.12** | 7.50 | 1.25 | 1310.00** | 675.00 | 9.50 |
| 43 | $GP_6$-219 | 100.00** | 40.00 | 5.00 | 8.14** | 3.53 | 1.50 | 8.84** | 0.40 | 0.15 | 16.98** | 3.93 | 1.65 | 1700.00** | 156.05 | 8.25 |
| 44 | $GP_6$-226 | 100.00** | 85.00** | 32.50 | 6.80** | 2.68 | 1.61 | 8.18** | 0.82 | 0.35 | 14.98** | 3.50 | 1.96 | 1500.00** | 298.20 | 63.60 |
| 45 | $GP_6$-236 | 100.00** | 67.50 | 55.00 | 9.27** | 3.46 | 1.20 | 4.92** | 0.67 | 0.45 | 14.19** | 4.13 | 1.65 | 1420.00** | 278.55 | 90.75 |
| 46 | $GP_6$-250 | 95.00** | 50.00 | 15.00 | 4.29 | 3.64 | 1.70 | 4.78** | 0.80 | 0.40 | 9.07** | 4.44 | 2.10 | 861.65** | 222.00 | 31.50 |
| 47 | $GP_6$-263 | 95.00** | 60.00 | 7.50 | 6.79** | 4.69 | 0.93 | 5.22** | 0.82 | 0.35 | 12.01** | 5.51 | 1.28 | 1140.00** | 330.60 | 9.63 |
| 48 | $GP_6$-271 | 100.00** | 90.00** | 65.00 | 5.32* | 10.32** | 3.84 | 5.52** | 0.79 | 0.53 | 10.84** | 11.11** | 4.37 | 1080.00** | 999.90** | 284.05 |
| 49 | $GP_6$-276 | 95.00** | 85.00** | 57.50 | 2.94 | 1.69 | 1.18 | 5.22** | 0.77 | 0.55 | 8.16** | 2.46 | 1.73 | 775.20** | 209.10 | 99.85 |
| 50 | $GP_6$-282 | 95.00** | 37.50 | 0.00 | 5.00 | 2.73 | 0.00 | 8.02** | 0.52 | 0.00 | 13.02** | 3.25 | 0.00 | 1240.00** | 122.65 | 0.00 |
| 51 | $GP_6$-286 | 92.50** | 67.50 | 40.00 | 6.20** | 9.73** | 3.64 | 6.28** | 1.15 | 0.67 | 12.48** | 10.88** | 4.31 | 1150.00** | 733.55** | 172.20 |
| 52 | $GP_6$-297 | 100.00** | 82.50** | 27.50 | 6.16** | 5.82** | 0.41 | 6.98** | 0.95 | 0.25 | 13.14** | 6.77 | 0.66 | 1310.00** | 559.30 | 18.55 |
| 53 | $GP_6$-303 | 85.00** | 0.00 | 0.00 | 4.38 | 0.00 | 0.00 | 7.40** | 0.00 | 0.00 | 11.78** | 0.00 | 0.00 | 1000.00** | 0.00 | 0.00 |
| 54 | $GP_6$-305 | 100.00** | 100.00** | 95.00** | 6.41** | 9.06** | 3.88 | 8.54** | 0.83 | 0.33 | 14.95** | 9.89** | 4.21 | 1500.00** | 989.00** | 399.95 |
| 55 | $GP_6$-310 | 100.00** | 92.50** | 87.50** | 7.96** | 2.89 | 1.83 | 11.04** | 1.20 | 0.72 | 19.00** | 4.09 | 2.55 | 1900.00** | 378.95 | 222.80 |
| 56 | $GP_6$-312 | 100.00** | 52.50 | 27.50 | 6.64** | 4.79 | 1.28 | 7.22** | 0.60 | 0.38 | 13.86** | 5.39 | 1.66 | 1390.00** | 283.90 | 45.65 |
| 57 | $GP_6$-313 | 90.00** | 22.50 | 0.00 | 6.34** | 0.91 | 0.00 | 7.50** | 0.30 | 0.00 | 13.84** | 1.21 | 0.00 | 1250.00** | 27.60 | 0.00 |
| 58 | $GP_6$-317 | 100.00** | 27.50 | 0.00 | 4.53 | 1.66 | 0.00 | 4.88** | 0.51 | 0.00 | 9.41** | 2.17 | 0.00 | 941.00** | 60.40 | 0.00 |
| 59 | $GP_6$-324 | 95.00** | 80.00** | 47.50 | 7.80** | 7.00** | 1.47 | 7.60** | 0.87 | 0.43 | 15.40** | 7.87** | 1.90 | 1460.00** | 629.60 | 90.20 |
| 60 | $GP_6$-325 | 100.00** | 87.50** | 62.50 | 3.82 | 7.46** | 4.02 | 3.04** | 0.30 | 0.16 | 6.86 | 7.76* | 4.18 | 686.00 | 678.60 | 261.55 |
| 61 | $GP_6$-326 | 100.00** | 100.00** | 80.00** | 3.30 | 5.25 | 2.32 | 6.80** | 2.31** | 0.75 | 10.10** | 7.56 | 3.07 | 1010.00** | 756.00** | 245.60 |
| 62 | $GP_6$-327 | 100.00** | 77.50** | 52.50 | 5.00 | 4.89 | 1.79 | 7.32** | 1.54 | 0.52 | 12.32** | 6.43 | 2.31 | 1230.00** | 497.80 | 120.95 |
| 63 | $GP_6$-331 | 100.00** | 82.50** | 57.50 | 4.34 | 5.58** | 1.96 | 3.50** | 0.82 | 0.40 | 7.84** | 6.40 | 2.36 | 784.00** | 527.60 | 136.60 |
| 64 | $GP_6$-332 | 95.00** | 80.00** | 47.50 | 13.00** | 6.37** | 2.69 | 6.20** | 0.64 | 0.33 | 19.20** | 7.01 | 3.02 | 1820.00** | 562.05 | 142.95 |
| 65 | $GP_6$-347 | 100.00** | 90.00** | 30.00 | 6.96** | 2.23 | 0.65 | 7.26** | 0.66 | 0.48 | 14.22** | 2.89 | 1.13 | 1420.00** | 260.58 | 33.75 |
| 66 | $GP_6$-358 | 95.00** | 32.50 | 12.50 | 5.76** | 3.98 | 2.43 | 4.66** | 0.67 | 0.62 | 10.42** | 4.65 | 3.05 | 989.90** | 151.40 | 37.55 |
| 67 | $GP_6$-366 | 95.00** | 82.50** | 77.50** | 5.41* | 6.66** | 4.34 | 5.62** | 1.55 | 0.90 | 11.03** | 8.21** | 5.24 | 1050.00** | 678.30 | 405.80 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | $GP_6$-370 | 90.00** | 90.00** | 67.50 | 11.42** | 5.08 | 1.82 | 5.34** | 0.89 | 0.49 | 16.76** | 5.97 | 2.31 | 1510.00** | 537.30 | 155.45 |
| 69 | $GP_6$-371 | 100.00** | 95.00** | 92.50** | 7.40** | 8.88** | 4.77 | 9.40** | 1.39 | 0.41 | 16.80** | 10.27** | 5.18 | 1680.00** | 975.65** | 478.55 |
| 70 | $GP_6$-374 | 80.00** | 72.50 | 52.50 | 5.12 | 3.68 | 1.73 | 4.30** | 1.12 | 0.67 | 9.42** | 4.80 | 2.40 | 753.60** | 348.05 | 125.75 |
| 71 | $GP_6$-384 | 100.00** | 72.50 | 52.50 | 13.80** | 6.00** | 2.40 | 8.84** | 0.71 | 0.38 | 22.64** | 6.71 | 2.78 | 2260.00** | 486.00 | 146.30 |
| 72 | $GP_6$-387 | 100.00** | 90.00** | 25.00 | 5.88** | 4.03 | 2.32 | 6.20** | 0.72 | 0.42 | 12.08** | 4.75 | 2.74 | 1210.00** | 427.50 | 69.70 |
| 73 | $GP_6$-400 | 90.00** | 77.50** | 42.50 | 7.48** | 4.57 | 2.37 | 9.44** | 0.92 | 0.73 | 16.92** | 5.49 | 3.10 | 1520.00** | 424.75 | 131.20 |
| 74 | $GP_6$-420 | 100.00** | 70.00 | 55.00 | 8.84** | 7.43** | 3.73 | 10.62** | 0.80 | 0.36 | 19.46** | 8.23** | 4.09 | 1950.00** | 576.10 | 224.20 |
| 75 | $GP_6$-424 | 100.00** | 95.00** | 92.50** | 4.21 | 7.88** | 2.80 | 9.34** | 1.57 | 0.61 | 13.55** | 9.45** | 3.41 | 1360.00** | 897.75** | 314.90 |
| 76 | $GP_6$-442 | 100.00** | 100.00** | 92.50** | 4.30 | 8.00** | 3.00 | 6.96** | 1.42 | 0.69 | 11.26** | 9.42** | 3.69 | 1130.00** | 942.00** | 340.85 |
| 77 | $GP_6$-451 | 95.00** | 77.50** | 37.50 | 5.83** | 2.74 | 1.22 | 6.10** | 0.83 | 0.51 | 11.93** | 3.57 | 1.73 | 1130.00** | 276.15 | 65.10 |
| 78 | $GP_6$-459 | 100.00** | 85.00** | 37.50 | 7.70** | 5.67** | 1.28 | 8.60** | 1.13 | 0.53 | 16.30** | 6.80 | 1.81 | 1630.00** | 578.00 | 68.35 |
| 79 | $GP_6$-511 | 100.00** | 60.00 | 10.00 | 6.36** | 4.87 | 1.00 | 5.48** | 0.61 | 0.38 | 11.84** | 5.48 | 1.38 | 1180.00** | 330.00 | 13.80 |
| 80 | $GP_6$-517 | 100.00** | 65.00 | 60.00 | 5.10 | 3.06 | 2.90 | 9.10** | 2.50** | 0.27 | 14.20** | 5.56 | 3.17 | 1420.00** | 360.70 | 190.20 |
| 81 | $GP_6$-534 | 95.00** | 22.50 | 0.00 | 3.40 | 1.38 | 0.00 | 7.18** | 0.15 | 0.00 | 10.58** | 1.53 | 0.00 | 1010.00** | 34.63 | 0.00 |
| 82 | $GP_6$-561 | 90.00** | 80.00** | 52.50 | 9.28** | 5.83** | 2.70 | 6.98** | 1.56 | 0.60 | 16.26** | 7.39 | 3.30 | 1460.00** | 591.20 | 173.75 |
| 83 | $GP_6$-570 | 100.00** | 75.00* | 55.00 | 6.28** | 5.41* | 2.50 | 8.22** | 1.23 | 0.57 | 14.50** | 6.64 | 3.07 | 1450.00** | 499.40 | 167.80 |
| 84 | $GP_6$-578 | 95.00** | 87.50** | 72.50 | 3.95 | 5.60** | 2.35 | 10.40** | 1.45 | 0.53 | 14.35** | 7.05 | 2.88 | 1360.00** | 617.50 | 208.40 |
| 85 | $GP_6$-579 | 100.00** | 90.00** | 50.00 | 6.49** | 2.37 | 0.90 | 5.32** | 0.30 | 0.20 | 11.81** | 2.67 | 1.10 | 1180.00** | 240.30 | 55.00 |
| 86 | $GP_6$-586 | 100.00** | 65.00 | 65.00 | 3.14 | 4.70 | 3.45 | 3.44** | 0.90 | 0.58 | 6.58 | 5.60 | 4.03 | 658.00 | 366.10 | 263.10 |
| 87 | $GP_6$-589 | 90.00** | 17.50 | 20.00 | 1.50 | 1.95 | 2.60 | 3.90** | 1.22 | 0.47 | 5.40 | 3.17 | 3.07 | 486.00 | 54.90 | 61.40 |
| 88 | $GP_6$-614 | 100.00** | 97.50** | 77.50** | 6.71** | 6.98** | 4.58 | 9.28** | 1.54 | 0.87 | 15.99** | 8.52** | 5.45 | 1600.00** | 831.00** | 422.10 |
| 89 | $GP_6$-615 | 100.00** | 67.50 | 5.00 | 6.82** | 3.07 | 0.60 | 8.46** | 0.76 | 0.15 | 15.28** | 3.83 | 0.75 | 1530.00** | 258.95 | 3.75 |
| 90 | $GP_6$-656 | 100.00** | 92.50** | 22.50 | 4.50 | 3.55 | 1.40 | 4.48** | 0.52 | 0.37 | 8.98** | 4.07 | 1.77 | 898.00** | 376.65 | 40.10 |
| 91 | $GP_6$-657 | 100.00** | 77.50** | 0.00 | 4.18 | 6.22** | 0.00 | 5.52** | 0.47 | 0.00 | 9.70** | 6.69 | 0.00 | 970.00** | 519.55 | 0.00 |
| 92 | $GP_6$-699 | 100.00** | 77.50** | 67.50 | 11.34** | 7.83** | 2.20 | 8.24** | 0.92 | 0.46 | 19.58** | 8.75** | 2.66 | 1960.00** | 679.05 | 179.95 |
| 93 | $GP_6$-714 | 100.00** | 85.00** | 80.00** | 3.39 | 6.95** | 6.00** | 5.00** | 2.05 | 0.48 | 8.39** | 9.00** | 6.48 | 839.00** | 763.00** | 518.40 |
| 94 | $GP_6$-734 | 96.67** | 92.50** | 55.00 | 6.91** | 8.59** | 4.77 | 7.94** | 1.21 | 0.40 | 14.85** | 9.80** | 5.17 | 1430.00** | 907.40** | 284.35 |
| 95 | $GP_6$-764 | 90.00** | 65.00 | 20.00 | 7.76** | 4.35 | 2.12 | 9.10** | 0.59 | 0.29 | 16.86** | 4.94 | 2.41 | 1520.00** | 322.40 | 48.10 |
| 96 | $GP_6$-792 | 100.00** | 85.00** | 37.50 | 7.34** | 6.28** | 1.57 | 6.50** | 0.94 | 0.36 | 13.84** | 7.22 | 1.93 | 1380.00** | 615.90 | 73.25 |
| 97 | $GP_6$-794 | 100.00** | 75.00* | 52.50 | 8.29** | 5.39* | 1.89 | 8.22** | 0.27 | 0.24 | 16.51** | 5.66 | 2.13 | 1650.00** | 424.50 | 112.20 |
| 98 | $GP_6$-819 | 100.00** | 37.50 | 5.00 | 9.12** | 1.12 | 0.90 | 9.90** | 0.25 | 0.17 | 19.02** | 1.36 | 1.07 | 1900.00** | 51.35 | 5.35 |
| 99 | $GP_6$-847 | 95.00** | 50.00 | 12.50 | 5.14 | 2.42 | 0.49 | 4.30** | 0.69 | 0.49 | 9.44** | 3.11 | 0.98 | 896.33** | 155.50 | 12.88 |
| 100 | $GP_6$-854 | 100.00** | 92.50** | 67.50 | 7.56** | 8.17** | 4.62 | 5.64** | 1.20 | 0.67 | 13.20** | 9.37** | 5.29 | 1320.00** | 866.15** | 357.75 |
| 101 | $GP_6$-861 | 90.00** | 30.00 | 0.00 | 9.52** | 2.54 | 0.00 | 7.12** | 0.19 | 0.00 | 16.64** | 2.73 | 0.00 | 1500.00** | 81.90 | 0.00 |
| 102 | $GP_6$-863 | 100.00** | 92.50** | 92.50** | 10.35** | 12.65** | 4.24 | 9.72** | 1.44 | 0.95 | 20.07** | 14.09** | 5.19 | 2010.00** | 1300.00** | 480.60 |
| 103 | $GP_6$-872 | 85.00** | 50.00 | 0.00 | 4.15 | 2.58 | 0.00 | 4.52** | 0.81 | 0.00 | 8.67** | 3.39 | 0.00 | 736.95** | 168.50 | 0.00 |
| 104 | $GP_6$-875 | 100.00** | 40.00 | 7.50 | 4.10 | 2.57 | 0.38 | 6.16** | 0.30 | 0.20 | 10.26** | 2.87 | 0.58 | 1030.00** | 114.60 | 4.63 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 105 | $GP_6$-883 | 100.00** | 72.50 | 12.50 | 8.82** | 3.85 | 1.13 | 7.50** | 0.95 | 0.35 | 16.32** | 4.80 | 1.48 | 1630.00** | 347.60 | 18.88 |
| 106 | $GP_6$-887 | 100.00** | 70.00 | 0.00 | 4.13 | 5.86** | 0.00 | 3.90** | 0.68 | 0.00 | 8.03** | 6.54 | 0.00 | 803.00** | 459.20 | 0.00 |
| 107 | $GP_6$-891 | 100.00** | 52.50 | 52.50 | 6.75** | 1.55 | 1.48 | 6.46** | 1.05 | 0.70 | 13.21** | 2.60 | 2.18 | 1320.00** | 136.70 | 115.15 |
| 108 | $GP_6$-899 | 95.00** | 65.00 | 10.00 | 8.44** | 3.65 | 0.30 | 8.20** | 0.44 | 0.25 | 16.64** | 4.09 | 0.55 | 1580.00** | 265.85 | 5.25 |
| 109 | $GP_6$-906 | 100.00** | 40.00 | 40.00 | 9.90** | 2.43 | 2.16 | 9.16** | 0.62 | 0.66 | 19.06** | 3.05 | 2.82 | 1910.00** | 122.75 | 111.70 |
| 110 | $GP_6$-912 | 100.00** | 87.50** | 72.50 | 5.70** | 8.00** | 3.88 | 5.94** | 1.11 | 0.33 | 11.64** | 9.11** | 4.21 | 1160.00** | 798.15** | 304.65 |
| 111 | $GP_6$-917 | 90.00** | 80.00** | 35.00 | 7.25** | 5.43** | 1.19 | 7.18** | 1.73 | 0.44 | 14.43** | 7.16 | 1.63 | 1300.00** | 572.80 | 58.70 |
| 112 | $GP_6$-951 | 80.00** | 25.00 | 15.00 | 6.80** | 3.97 | 1.75 | 6.68** | 0.49 | 0.25 | 13.48** | 4.46 | 2.00 | 1080.00** | 109.80 | 29.25 |
| 113 | $GP_6$-952 | 100.00** | 65.00 | 10.00 | 7.72** | 3.10 | 1.58 | 7.70** | 0.56 | 0.33 | 15.42** | 3.66 | 1.91 | 1540.00** | 237.90 | 19.05 |
| 114 | GP6-953 | 100.00** | 70.00 | 20.00 | 13.90** | 7.00** | 1.59 | 8.50** | 0.60 | 0.33 | 22.40** | 7.60 | 1.92 | 2240.00** | 532.60 | 37.53 |
| 115 | $GP_6$-961 | 80.00** | 42.50 | 37.50 | 10.57** | 4.73 | 2.74 | 6.66** | 1.26 | 0.49 | 17.23** | 5.99 | 3.23 | 1380.00** | 253.95 | 120.53 |
| 116 | $GP_6$-965 | 100.00** | 97.50** | 65.00 | 9.30** | 5.70** | 3.99 | 10.60** | 0.70 | 0.46 | 19.90** | 6.40 | 4.45 | 1990.00** | 623.15 | 288.90 |
| 117 | $GP_6$-967 | 100.00** | 90.00** | 80.00** | 3.82 | 5.43** | 3.09 | 7.52** | 1.19 | 0.52 | 11.34** | 6.62 | 3.61 | 1130.00** | 595.80 | 288.80 |
| 118 | $GP_6$-969 | 100.00** | 90.00** | 90.00** | 4.36 | 5.23 | 2.23 | 8.20** | 1.72 | 0.58 | 12.56** | 6.95 | 2.81 | 1260.00** | 625.50 | 252.90 |
| 119 | $GP_6$-990 | 100.00** | 45.00 | 15.00 | 5.18 | 1.69 | 1.00 | 8.30** | 0.70 | 0.45 | 13.48** | 2.39 | 1.45 | 1350.00** | 107.55 | 21.75 |
| 120 | $GP_6$-1001 | 95.00** | 77.50** | 45.00 | 11.63** | 4.49 | 1.96 | 8.88** | 0.77 | 0.56 | 20.51** | 5.26 | 2.52 | 1950.00** | 408.60 | 114.70 |
| 121 | $GP_6$-1020 | 100.00** | 82.50** | 17.50 | 8.39** | 3.83 | 2.78 | 6.42** | 0.44 | 0.33 | 14.81** | 4.27 | 3.10 | 1480.00** | 353.00 | 53.63 |
| 122 | $GP_6$-1023 | 95.00** | 85.00** | 55.00 | 5.82** | 7.60** | 1.76 | 9.56** | 1.35 | 0.40 | 15.38** | 8.95** | 2.16 | 1460.00** | 760.75** | 118.80 |
| 123 | $GP_6$-1026 | 90.00** | 67.50 | 0.00 | 3.61 | 1.17 | 0.00 | 5.50** | 0.42 | 0.00 | 9.11** | 1.59 | 0.00 | 819.90** | 107.30 | 0.00 |
| 124 | $GP_6$-1037 | 95.00** | 77.50** | 32.50 | 6.45** | 9.95** | 3.10 | 11.00** | 1.04 | 0.45 | 17.45** | 10.99** | 3.55 | 1660.00** | 852.60** | 116.10 |
| 125 | $GP_6$-1047 | 100.00** | 90.00** | 52.50 | 3.31 | 2.90 | 0.38 | 6.48** | 0.73 | 0.31 | 9.79** | 3.63 | 0.69 | 979.00** | 326.70 | 36.40 |
| 126 | $GP_6$-1060 | 100.00** | 95.00** | 80.00** | 4.18 | 6.96** | 3.11 | 7.20** | 0.97 | 0.68 | 11.38** | 7.93** | 3.79 | 1140.00** | 753.35** | 303.20 |
| 127 | $GP_6$-1063 | 100.00** | 90.00** | 20.00 | 5.74** | 3.60 | 1.55 | 9.72** | 0.49 | 0.33 | 15.46** | 4.09 | 1.88 | 1550.00** | 368.10 | 37.60 |
| 128 | $GP_6$-1072 | 100.00** | 80.00** | 75.00* | 9.40** | 5.72** | 5.80** | 11.24** | 0.85 | 0.51 | 20.64** | 6.57 | 6.31 | 2060.00** | 526.45 | 473.25 |
| 129 | $GP_6$-1075 | 100.00** | 90.00** | 57.50 | 3.58 | 5.52** | 2.77 | 6.64** | 1.15 | 0.37 | 10.22** | 6.67 | 3.14 | 1020.00** | 600.30 | 181.20 |
| 130 | $GP_6$-1089 | 100.00** | 12.50 | 0.00 | 9.47** | 0.68 | 0.00 | 6.40** | 0.48 | 0.00 | 15.87** | 1.16 | 0.00 | 1590.00** | 14.68 | 0.00 |
| 131 | $GP_6$-1101 | 100.00** | 82.50** | 40.00 | 5.41* | 7.70** | 3.06 | 5.08** | 1.15 | 0.67 | 10.49** | 8.85** | 3.73 | 1050.00** | 728.90** | 149.20 |
| 132 | $GP_6$-1102 | 100.00** | 82.50** | 75.00* | 4.70 | 7.20** | 4.35 | 5.20** | 1.75 | 0.71 | 9.90** | 8.95** | 5.06 | 990.00** | 739.50** | 379.10 |
| 133 | $GP_6$-1114 | 97.50** | 75.00* | 17.50 | 6.28** | 4.18 | 0.60 | 2.80** | 0.51 | 0.27 | 9.08** | 4.69 | 0.87 | 885.30** | 351.70 | 15.75 |
| 134 | $GP_6$-1117 | 100.00** | 87.50** | 35.00 | 3.81 | 5.83** | 1.22 | 4.46** | 1.36 | 0.40 | 8.27** | 7.19 | 1.62 | 827.00** | 630.05 | 56.70 |
| 135 | $GP_6$-1127 | 100.00** | 57.50 | 7.50 | 5.66** | 3.47 | 0.35 | 5.36** | 0.47 | 0.50 | 11.02** | 3.94 | 0.85 | 1100.00** | 227.25 | 6.50 |
| 136 | $GP_6$-1135 | 100.00** | 57.50 | 0.00 | 11.40** | 2.70 | 0.00 | 7.80** | 0.49 | 0.00 | 19.20** | 3.19 | 0.00 | 1920.00** | 183.10 | 0.00 |
| 137 | $GP_6$-1150 | 90.00** | 50.00 | 17.50 | 7.96** | 4.33 | 1.97 | 9.10** | 0.74 | 0.63 | 17.06** | 5.07 | 2.60 | 1540.00** | 253.50 | 45.10 |
| 138 | $GP_6$-1207 | 100.00** | 90.00** | 20.00 | 11.36** | 5.94** | 0.93 | 9.24** | 0.83 | 0.30 | 20.60** | 6.77 | 1.23 | 2060.00** | 609.30 | 25.25 |
| 139 | $GP_6$-1217 | 95.00** | 87.50** | 10.00 | 8.17** | 3.20 | 0.13 | 9.56** | 0.93 | 0.18 | 17.73** | 4.13 | 0.31 | 1680.00** | 360.85 | 3.05 |
| 140 | $GP_6$-1227 | 90.00** | 32.50 | 12.50 | 4.37 | 2.28 | 0.10 | 2.82** | 0.45 | 0.20 | 7.19 | 2.73 | 0.30 | 647.10 | 88.50 | 3.75 |
| 141 | $GP_6$-1228 | 95.00** | 75.00* | 67.50 | 14.24** | 8.45** | 3.22 | 8.92** | 1.64 | 0.33 | 23.16** | 10.09** | 3.55 | 2200.00** | 756.20** | 239.20 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 142 | $GP_6$-1254 | 90.00** | 80.00** | 62.50 | 10.60** | 10.17** | 6.37** | 10.30** | 1.42 | 0.75 | 20.90** | 11.59** | 7.12 | 1880.00** | 927.20** | 445.25 |
| 143 | $GP_6$1350 | 77.50** | 25.00 | 12.50 | 3.67 | 1.29 | 1.19 | 3.98** | 0.71 | 0.40 | 7.65* | 2.00 | 1.59 | 592.75 | 49.50 | 20.53 |
| 144 | $GP_6$1436 | 77.50** | 47.50 | 25.00 | 7.20** | 4.99 | 1.00 | 5.38** | 0.54 | 0.28 | 12.58** | 5.53 | 1.28 | 974.70** | 263.65 | 31.30 |
| 145 | $GP_6$1450 | 100.00** | 15.00 | 0.00 | 5.18 | 0.52 | 0.00 | 7.92** | 0.28 | 0.00 | 13.10** | 0.80 | 0.00 | 1310.00** | 11.93 | 0.00 |
| 146 | $GP_6$-1468 | 100.00** | 87.50** | 32.50 | 3.26 | 8.05** | 2.53 | 4.58** | 0.80 | 0.42 | 7.84** | 8.85** | 2.95 | 784.00** | 774.80** | 96.30 |
| 147 | $GP_6$-1477 | 100.00** | 80.00** | 32.50 | 2.80 | 3.00 | 1.87 | 4.56** | 0.89 | 0.45 | 7.36 | 3.89 | 2.32 | 736.00** | 312.53 | 75.55 |
| 148 | $GP_6$1482 | 90.00** | 72.50 | 30.00 | 3.59 | 4.29 | 2.32 | 5.80** | 1.71 | 1.19 | 9.39** | 6.00 | 3.51 | 845.10** | 435.35 | 106.75 |
| 149 | $GP_6$-1509 | 90.00** | 62.50 | 35.00 | 7.75** | 6.70** | 2.37 | 7.70** | 1.20 | 0.48 | 15.45** | 7.90** | 2.85 | 1390.00** | 493.20 | 101.00 |
| 150 | $GP_6$-1518 | 100.00** | 87.50** | 35.00 | 11.34** | 6.11** | 0.97 | 7.84** | 0.84 | 0.44 | 19.18** | 6.95 | 1.41 | 1920.00** | 608.85 | 49.35 |
| 151 | $GP_6$-1533 | 90.00** | 75.00* | 57.50 | 5.64** | 6.79** | 3.29 | 6.44** | 0.92 | 0.51 | 12.08** | 7.71* | 3.80 | 1090.00** | 578.25 | 219.00 |
| 152 | $GP_6$-1561 | 100.00** | 82.50** | 35.00 | 7.95** | 6.05** | 1.53 | 7.60** | 1.11 | 0.42 | 15.55** | 7.16 | 1.95 | 1560.00** | 591.10 | 69.00 |
| 153 | $GP_6$-1573 | 100.00** | 95.00** | 45.00 | 5.58** | 5.34* | 3.06 | 5.36** | 0.62 | 0.38 | 10.94** | 5.96 | 3.44 | 1090.00** | 564.60 | 153.40 |
| 154 | $GP_6$-1576 | 100.00** | 95.00** | 85.00** | 4.36 | 5.21 | 2.19 | 4.08** | 0.92 | 0.51 | 8.44** | 6.13 | 2.70 | 844.00** | 578.30 | 229.50 |
| 155 | $GP_6$-1588 | 95.00** | 52.50 | 7.50 | 6.34** | 3.43 | 1.20 | 8.84** | 0.77 | 0.70 | 15.18** | 4.20 | 1.90 | 1440.00** | 221.40 | 14.25 |
| 156 | $GP_6$-1595 | 100.00** | 75.00* | 65.00 | 5.28* | 2.72 | 2.15 | 7.46** | 0.73 | 0.64 | 12.74** | 3.45 | 2.79 | 1270.00** | 257.40 | 181.35 |
| 157 | $GP_6$-1616 | 100.00** | 95.00** | 70.00 | 4.29 | 3.64 | 2.85 | 5.30** | 1.05 | 0.28 | 9.59** | 4.69 | 3.13 | 959.00** | 444.10 | 219.10 |
| 158 | $GP_6$-1665 | 100.00** | 95.00** | 27.50 | 7.44** | 2.59 | 0.50 | 6.40** | 0.56 | 0.55 | 13.84** | 3.15 | 1.05 | 1380.00** | 299.90 | 29.25 |
| 159 | $GP_6$-1725 | 100.00** | 85.00** | 60.00 | 6.46** | 6.27** | 3.91 | 4.62** | 1.03 | 0.70 | 11.08** | 7.30 | 4.61 | 1110.00** | 620.50 | 277.15 |
| 160 | $GP_6$-2255 | 100.00** | 100.00** | 95.00** | 8.58** | 7.81** | 3.64 | 6.92** | 1.44 | 0.83 | 15.50** | 9.25** | 4.47 | 1550.00** | 925.00** | 424.65 |
| Range | | | 12.50-100 | 5-95 | | 0.52-12.65 | 0.10-6.56 | | 0.25-2.50 | 0.12-1.20 | | 0.80-14.09 | 0.30-7.76 | | | |
| Mean | | 96.35 | 72.20 | 40.33 | 6.22 | 4.61 | 1.94 | 6.50 | 0.89 | 0.42 | 12.72 | 5.50 | 2.36 | 1228.00 | 428.06 | 128.63 |
| CD 5% | | | 7.13 | | | 0.48 | | | 0.06 | | | 0.65 | | | 81.77 | |
| CD 1% | | | 9.39 | | | 0.63 | | | 0.08 | | | 0.86 | | | 107.63 | |

Table 4. Comparative performance of 160 sunflower genotypes under control and moisture stress conditions using stress inducing chemical PEG-6000 at 20 and 25 per cent concentrations

| Characters | Overall mean values GXT interaction | | | Changes in mean value | | Percent change in mean | | No. of significant genotypes recorded out of 160 | | | Promising genotypes identified for moisture stress tolerance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control (Non-stress) | | PEG-6000 (Moisture stress) | | | | | | | | |
| | **0 %** | **20 %** | **25 %** | **20 %** | **25 %** | **20 %** | **25 %** | **0 %** | **20 %** | **25 %** | CMS 857B, CMS 104B,CMS 378B (3) R-78, R-12-2, R 630, GM 71R (4) $GP_6$-305, $GP_6$-371, $GP_6$-118, $GP_6$-714, $GP_6$-1072, $GP_6$-325, $GP_6$-2255, $GP_6$-424, $GP_6$-442, $GP_6$-863, $GP_6$-11, $GP_6$-969, $GP_6$-310, $GP_6$-211, $GP_6$-1576, $GP_6$-326, $GP_6$-967, $GP_6$-1060, $GP_6$-366, $GP_6$-614, $GP_6$-54, $GP_6$-1102, $GP_6$-1254, $GP_6$-1616, $GP_6$-420, $GP_6$-219, $GP_6$-517, $GP_6$-912, $GP_6$-734, $GP_6$-586 and $GP_6$-589 (36) |
| Germination per cent | 96.35 | 72.20 | 40.33 | -24.15 | -56.02 | -25.06 | -58.14 | 159 | 94 | 24 | |
| Root length (cm) | 6.22 | 4.61 | 1.94 | -1.61 | -4.28 | -25.88 | -68.81 | 96 | 62 | 5 | |
| Shoot length (cm) | 6.50 | 0.89 | 0.42 | -5.61 | -6.08 | -86.30 | -93.53 | 160 | 2 | 0 | |
| Seedling length (cm) | 12.72 | 5.50 | 2.36 | -7.22 | -10.36 | -56.76 | -81.45 | 143 | 33 | 1 | |
| Seed vigour | 1228 | 428.06 | 128.63 | -799.94 | -1099.37 | -65.14 | -89.50 | 147 | 26 | 2 | |

## LITERATURE


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# GENOME-WIDE ASSOCIATION OF OIL YIELD PLASTICITY TO DROUGHT, NITROGEN AND CHILLING STRESSES IN SUNFLOWER

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## ABSTRACT

To face the climate change, the plasticity of genome is a great advantage that plant breeders could build on in order to adapt varieties to new environments. A way to accelerate the adaptation is by the discovery of new alleles involved in the plasticity responses and their introgression in elite varieties. So we conducted a genome wide association study (GWAS) in plasticity responses face to multiple abiotic stresses. We characterized 14 environments by their levels face to four abiotic stresses: nitrogen, drought, chilling and heat, thanks to the SUNFLO crop model. Three known varieties, used as controls and observed in the 14 environments, allowed computing the stress felt during key periods of the growth development. Among the 56 stress indices computed by SUNFLO, the best model to fit the oil yield, regarding the AIC criteria averaged on the three above control varieties, contained only three stresses: the nitrogen during the whole growth period, the drought from sowing to filling and the chilling before flowering. The observed oil yield of a panel of 371 sunflower lines was regressed by linear norm reaction model with the three stress indices of the best model. The slope of each stress norm reaction was used as plasticity phenotypes. Association mapping was based on a set of 65,534 SNPs with MAF >0.05 using the usual mixed model of association, including the maintainer or restorer status as fixed effect and the Alike in State relatedness matrix for the polygenic random term. A forward approach was performed to detect multiple associated SNPs. Homology study of detected SNPs with the annotated genes of Arabidopsis completed the analyses. The results concerning the plasticity face to chilling will be detailed in this talk

**Key Words :** Plasticity, multi-stress, abiotic, GWAS

# BREEDING FOR SUNFLOWER HYBRIDS ADAPTED TO CLIMATE CHANGE: THE SUNRISE COLLABORATIVE AND MULTI-DISCIPLINARY PROJECT

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## ABSTRACT

In the context of climate change, an increased variability is expected in timing and amount of water available for crop production. For sunflower crop, yield losses of 10 to 30 % have been predicted at 2030 horizon in Europe. During the past 10 years, genetic progress was lower than expected to improve yield, which imposes to the sunflower community to re-invest current breeding resources and methodologies. To reach high and stable yields across a wide range of environments a French project of 8 years, named 'SUNRISE' (SUNflower Resources to Improve yield Stability in a changing Environment) and supported by the French National Research Agency, is gathering 9 public and 7 private partners since 2012. It associates several approaches: (i) the sequencing and genotyping of the genetic diversity among cultivated and wild sunflowers, (ii) the development of appropriate and high-throughput phenotyping strategies to characterize the molecular, physiological and agronomical responses to variation of the abiotic environment, (iii) the discovery through genome-wide association, linkage mapping and genomic selection of the genetic factors involved in those responses, (iv) the integration of this genetic knowledge into a crop model (SUNFLO) to test in silico G by E interactions and design promising ideotypes in future environments, and finally (v) the evaluation of the outputs for the breeding sector and the transfer of knowledge to agriculture. This partnership will ensure that the developed knowledge, resources and methods will be translated into products and varieties supporting the adaptation of the agriculture to societal and ecological challenges.

**Key Words :** drought, genetic resources, breeding, crop model, ideotype, genome

# CONTROL OF *VERTICILLIUM DAHLIAE* CAUSING SUNFLOWER WILT USING *BRASSICA* COVER CROPS

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## ABSTRACT

Since 2010, sunflower in France has been severely affected by a vascular wilt disease caused by *Verticillium dahliae*. Disease is widespread and causes significant damage up to 30% yield loss. *V. dahliae* is a soil-borne fungus living in roots able to survive in the absence of a host in the form of microsclerotia (MS). *Brassica* crops used as cover crops can naturally suppress soilborne pathogen viability. This fumigation activity has been linked to volatile isothiocyanates (ITCs) released from glucosinolates (GSLs). In this study, *Brassica* cover crops of white and brow mustard, radish, rape and rapeseed were evaluated for their ability to reduce the viability and development of *V. dahliae*. Cultivars were selected by GSL side-chain and concentration, and *V. dahliae* strain for its aggressiveness on sunflower. Biofumigation was assessed in a laboratory assay. MS and developed *V. dahliae* on growing media were exposed for 20 days to volatile compounds released by fresh or freeze ground plant tissues. The toxicity of ITCs-GSLs on *V. dahliae* was assessed by the area under fungus progress curve relating its development on the media. The five *Brassica* reduced the development and germination of *V. dahliae* by 90% (brown-mustard) to 63% (radish), and the development by 90% (rape) to 69% (white-mustard) compared to the control in absence of tissues. Aliphatic GSLs in brown mustard and rape, and indole GSLs in rape and radish may explain the strong reduction of *V. dahliae* development and viability respectively. These results indicate that *Brassica* have potential for use as cover crops for the control of soilborne disease problems and sunflower wilt.

**Keywords** *Verticillium dahliae*, Microsclerotia, Biofumigation, Cover crops, *Brassica* crops, glucosinolates, Sunflower.

## INTRODUCTION

*Verticillium dahlia* Kleb. is a destructive and vascular soil-borne fungus that infects many economically major agricultural crops and ornamental plants all around the world (Schnathorst, 1981; Pegg and Brady, 2002). In France, since 2010, sunflower (*Helianthus Annuus*) has been severely affected by this pathogen causing significant damage up to 30% yield loss (Mestries and Lecomte, 2012). Symptoms of sunflower Verticillium wilt appears around flowering, first on the lower leaves and move towards upper leaves. On leaf, brown necrosis surround by a yellow halo, vascular discoloration and wilting of sunflower are observed (Wilhelm, 1956). Verticillium wilt is difficult to control because the pathogen can survive in the soil as microsclerotia (MS), its resting structure, for nearly 13 years even in the absence of a suitable host (Bruehl, 1987 ; Griffiths, 1970). Thus, MS are regarded as the primary targets to control Verticillium wilt (Hawke and Lazarovits, 1994).

Since the prohibition of effective but harmful chemical fumigants as methyl bromide, techniques to manage *V. dahliae* in sunflower are limited and not very effective. Alternative methods including sustainable disease control options for managing soilborne fungus are needed (Davis et al., 2010 ; Ochiai et al., 2007 ; Rowe and Powelson, 2002). Thus,

biofumigation, performed by the incorporation of fresh biomass from *Brassica* plants into the soil, appears as an alternative promising method (Kirkegaard et al., 1993 ; Angus et al., 1994 ; Kirkegaard et al., 1998 ; Kirkegaard et al., 2000 ; Matthiessen and Kirkegaard, 2006 ; Larkin and Griffin, 2007 ; Njoroge et al., 2008; Omirou et al., 2011). *Brassica* crops used as cover crops have disease-suppressive effects against soilborne population of fungal pathogens, nematodes and weeds (Brown and Morra, 1995, 1997 ; Buskov et al., 2002 ; Mojtahedi, 1993 ; Olivier et al., 1999 ; Sarwar et al., 1998). It is based on the high concentration of glucosinolates (GSLs) which are secondary metabolites structurally categorized as aliphatic, aromatic, and indole GSLs (Brown and Morra, 1997 ; Fahey et al., 2001 ; Omirou et al., 2011). GSLs are biologically inactive molecules, but after tissues disruption, GSL are hydrolyzed by myrosinase to volatile compounds like indoles, nitriles, thiocyanates and isothiocyanates (ITCs). Among those, ITCs have a biocidal activity and are the most toxic for soil-borne pathogens (Chew, 1988 ; Fenwick and Heaney, 1983 ; Mithen, 2001 ; Omirou et al., 2011 ; Rosa, 1997 ; Sarwar et al., 1998). However, the profile, concentration and distribution of GSLs – ITCs varies greatly within Brassica species, plant tissues and even among cultivars (Kirkegaard et al., 1998 ; Mithen, 1992).

Few studies have investigated the potential of GSLs-containing brassicaceous cover crops for suppression of *V. dahliae*. Additionally, the role of ITC-related biofumigation often cannot be interpreted because no information is provide on the type or the concentration of GSLs present in the used biomass. Thus, predictions of the biofumigation potential of different *Brassica* species to *V. dahliae* based on GSLs concentration needs to be confirmed to evaluate the incidence of their potential biocidal activity *in vitro*. The adoption of biofumigation seems most likely to proceed if it is specified to suit the target pests and production systems. This preliminary study aimed at evaluating the potential biofumigation effects of *Brassica* cover crops on *V. dahliae* - sunflower pathosytem. The objective of this study was (1) to assess the potential biofumigation effects of five *Brassica* cover crops by following *in vitro* the relative toxicity of relevant GSLs on *V. dahliae* development and MS formation (2) to identify the most effective Brassica crops for *V. dahliae* control in future field trials.

## MATERIALS AND METHODS

*Production of biomass, sampling and sample preparation*

Seeds of 5 different cultivars of brown mustard (*Brassica juncea* cv Etamine) (100 pl/m²), white mustard (*Sinapis alba* cv. Abraham) (100 pl/m²), radish (*Rhaphanus sativus* cv. Anaconda) (80 pl/m²), rape (*Brassica rapa* cv. Avalon) (80 pl/m²) and rapeseed (*Brassica napus* cv. Mosa) (80 pl/m²) were sown on February 2015 in 5 trays (0.6 m²; 50 cm depth) filled with potting compost into the greenhouse of INRA, Auzeville (Haute-Garonne, France). These five cultivars were selected among 22 after a field trial in 2014 for their GSL profiles and concentrations in the shoot and root tissue for each crop.

Photoperiod, temperature and air humidity were controlled in the greenhouse. A 13h photoperiod was applied from plant emergence to flowering stage with 400W High Pressure Sodium vapor lamp (SON-T AGRO, Philips). Supplemental lighting was turned off when global radiation was above 250 W/m². The temperature in the greenhouse was maintained at 13°C ± 3°C. Plants were fertilized with two applications of NPK (24 kg N, 28 kg P, 28 kg K /ha) and $SO_3$ (40 kg/ha) to provide nutriments for GSL synthesis in the plant. Plants were irrigated regularly to maintain adequate soil moisture until flowering. Powdery mildew (*Erysiphe cichoracearum*) was treated by triticonazole (POLYSOINS ULTRA SPRAY, Scotts France SAS) at 0.15 g /L.

The relative production of GSLs in the tissues usually reach a maximum around flowering (Sarwar and Kirkegaard, 1998). Accordingly, brown and white mustard, and radish were sampled at mid-flowering at 51 days after sawing (DAS), and rape and rapeseed at 61 DAS. Roots (RB) and shoots (SB) were sampled, washed and then separated. RB and SB of each cultivar were grinded separately using a ELIET primo mill. The mill was rinsed between each sample. Samples of a particle size <0.5 cm were either used fresh for the in-vitro assay or stored in sealed bags and immediately frozen and stored at -80 °C until processing.

*Isolation and analysis of the desulfated GSL*

Frozen plant materials were freeze dried and ground as fine as possible with a Tetsch MM 300 mixer mill at 30 Hz for 1 min. Aliquot of 50 mg were weighed in 2.0 ml Eppendorf tubes after witch 1ml 70% MeOH was added to the samples and boiled it for 10 min at 90°C. After boiling, samples were placed for 15 min in an ultrasonic bath and centrifuged at 6500 rpm for 10 min. The supernatant was added to 0,5ml DEAE Sephadex A-25 column. The pellet was kept, washed twice with 1ml 70% MeOH, vortex and placed in the ultrasonic bath for 15 min. After centrifugation at 6500 rpm for 10 min the supernatant was added to the same column. The column was washed twice with 1ml 70% MeOH, once with 1ml MilliQ and twice with 1ml 20mM NaOAC buffer (pH 5.5). Thereafter, 20 µl of aryl sulfatase (Sigma type H-1 of *Helix pomatia*) was added to the columns and flushed down with 50 µL NaOAc buffer (pH 5.5). The columns were covered with aluminium foil and incubated over night at room temperature. The next day, the resulting desulphoglucosinolates were elute from the column with 2 times 0,75ml MilliQ water. The elution was frozen with liquid nitrogen, freeze dried, dissolved in 1ml MilliQ water and measured on the HPLC (de Graaf et al., 2015).

*Verticillium dahliae isolates and inoculum production*

A *V. dahliae* strain obtained from one microsclerotia (MS) was used in this study and selected for its aggressiveness among 8 strains. The strain was isolated from an infected sunflower residue in a field located in Verfeil (Haute-Garonne, France), close to the trial site, and showing severe Verticillium wilt, root dislocation and high production of MS. Inoculum was plated on Petri dishes containing potato dextrose agar (PDA, Difco) (39 g/l, 150 mg of streptomycin, pH 6) and grown at 25 ± 1 °C in the dark. For *in vitro* assay, the fungus was either used developed (DV) after 10 days growing on PDA and containing mycelium, spores and MS, or as an agar plug of MS (MSV) transplanted on PDA plate.

*Evaluation of biofumigation potential of Brassica plant biomass on V. dahliae.*

*In vitro* assay was developed to evaluate the biofumigation potential of RB, SB and a mix of root and shoot biomass (RSB), fresh or frozen, of white and brown mustard, radish, rape and rapeseed to control *V. dahliae*. The capacity of biofumigant cover crop on *V. dahliae* mycelial growth and MS germination was tested on DV and MSV fungus. For each fungus treatment, jars containing either 5 g of grinded RB, SB or RSB (4 g SB + 1g RB), fresh or frozen, were closed with inverted PDA petri containing DV or MS fungus. Each treatment were replicated 5 times corresponding to 300 jars in total. Control jars with inverted DV and MSV were prepared but no biofumigant material was added. Control were replicated 15 times. Jars were sealed with Parafilm® and incubated at 24 °C in the dark for 21 days. The radial growth of DV and MSV fungus was determined weakly.

*Data analyses*

Area under the fungus development progress curve (AUDPC) was calculated based on the weakly measurement of the fungus growth diameter on Petri dish. AUDPC was calculated according to the equation of Campbell and Madden (1990):

$$AUDPC = \sum_{i}^{n-1} (y_i + y_{i+1})/2 * (t_{i+1} - t_i)$$

where *n* is the number of measurement, *y* the growth diameter of the fungus, and *t* the days between each evaluation. Variables were analyzed by analysis of variance (ANOVA) using Statgraphics Plus 5.1 statistical software (Rockville, MA, USA) with replicate as a random variable. For each ANOVA, homogeneity of variance by Levene's test (confidence level of 0.95) and the normality of the residuals by the Shapiro-Wilks test (confidence level of 0.95) were conducted. When the F ratio was significant ($P < 0.05$), differences between treatment means were determined using protected least significant difference (LSD).

## RESULTS

### *GSL concentration in Brassica crops*

The GSLs profile and concentration in the shoot and root tissues of each biofumigant crops are shown in the Table 1. The contrasting GSL profiles between the five Brassica species, and within RB and SB is significant. The GSL profiles of white mustard and rape had significant aromatic GSLs with main concentration of sinalbin in SB and gluconasturtiin in BR respectively. The brown mustard GSLs profiles were dominated by sinigrin (aliphatic GSL) in BS and radish by unknown indole 16.3 (indole GSL) in RB. The GSLs profile of the rape biomass was more diverse including appreciable concentrations of glucobrassicanapin (aliphatic GSL), gluconasturtiin (aromatic GSL) and neoglucobrassicin (indole GSL) mainly in RB.

**Table 1.** Type and mean concentration of main glucosinolates in the shoot (SB) and root (RB) in the tissues of *Brassicas* biofumigant crop varieties

| | Glucosinolate concentration (µmol.g$^{-1}$ dry weight tissue) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | White Mustard | | Brown Mustard | | Rape | | Rapeseed | | Radish | |
| | SB | RB | SB | RB | SB | RB | SB | RB | SB | RB |
| ***Aliphatic*** | | | | | | | | | | |
| Sinigrin | 1,3 | 0,0 | 42,1 | 3,6 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Glucoerucin | 3,0 | 0,1 | 0,0 | 0,0 | 0,0 | 0,6 | 0,0 | 1,1 | 0,0 | 0,0 |
| Glucoraphanin | 0,2 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,1 | 0,0 | 0,0 | 0,0 |
| Gluconapin | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,2 | 0,1 | 0,0 | 0,0 |
| Progoitrin | 0,0 | 0,0 | 0,0 | 0,0 | 0,9 | 2,6 | 0,1 | 0,3 | 0,0 | 0,0 |
| Glucobrassicanapin | 0,0 | 0,0 | 0,0 | 0,0 | 1,9 | 4,2 | 0,3 | 0,2 | 0,0 | 0,0 |
| ***Aromatic*** | | | | | | | | | | |
| Gluconapoleiferin | 0,0 | 0,1 | 0,0 | 0,0 | 1,6 | 3,0 | 0,0 | 0,1 | 0,0 | 0,0 |
| Gluconasturtiin | 0,1 | 2,8 | 0,0 | 2,4 | 0,0 | 13,4 | 0,0 | 15,2 | 0,0 | 0,0 |
| Glucotropaeolin | 2,8 | 0,9 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Sinalbin | 15,1 | 4,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,1 | 0,0 | 0,0 |
| ***indole*** | | | | | | | | | | |
| 4-hydroxyglucobrassicin | 0,0 | 0,1 | 0,0 | 0,1 | 0,5 | 1,0 | 0,0 | 0,1 | 0,0 | 0,0 |
| Glucobrassicin | 0,0 | 0,1 | 0,2 | 0,0 | 0,7 | 0,8 | 0,7 | 0,4 | 5,2 | 0,1 |
| 4-methoxyglucobrassicin | 0,1 | 0,2 | 0,0 | 0,0 | 0,5 | 1,1 | 0,1 | 0,2 | 0,3 | 0,2 |
| Neoglucobrassicin | 0,0 | 0,4 | 0,1 | 0,4 | 1,5 | 4,9 | 0,8 | 2,2 | 0,0 | 0,0 |
| Unknown indole 16.3 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 7,4 | 41,6 |

### *Biofumigation potential of plant biomass on V. dahliae development*

Results from the *in vitro* assays to evaluate the incidence of different biofumigant crops showed that the *V. dahliae* development or germination, measured by the AUDPC, was significantly ($P < 0.05$) affected both for the fungus developed (DV) or the plug of microsclerotia (MSV) compared with the nonamended control (Fig. 1A and B). The AUDPC

was reduced by 63 to 90 % according to the species and a stronger impact on MSV fungus than DV was observed.

The biofumigant potential of the five *Brassica* toward *V. dahliae* differed according to the fungus stage (DV /MSV) (Fig. 1). From DV fungus, brown mustard and rape were more effective and radish was less effective to reduce mycelial growth of *V. dahliae* (Fig. 1A). From MSV, rape and radish were more effective than white mustard to reduce the MS germination of *V. dahliae* (Fig. 1B).

Regarding the type of biomass, there was no significant ($P > 0.05$) difference of RB, SB or RSB on fungus development for the different cover crop except for SB of brown mustard and RB of rape in DV, and for RB of the radish in MSV that reduced significantly ($P < 0.05$) the progression of the fungus. Because there was no significant effect of the cover crop biomass conditioning (fresh or frozen) on fungus development (DV and MSV), statistical analyses were performed on pooled data.

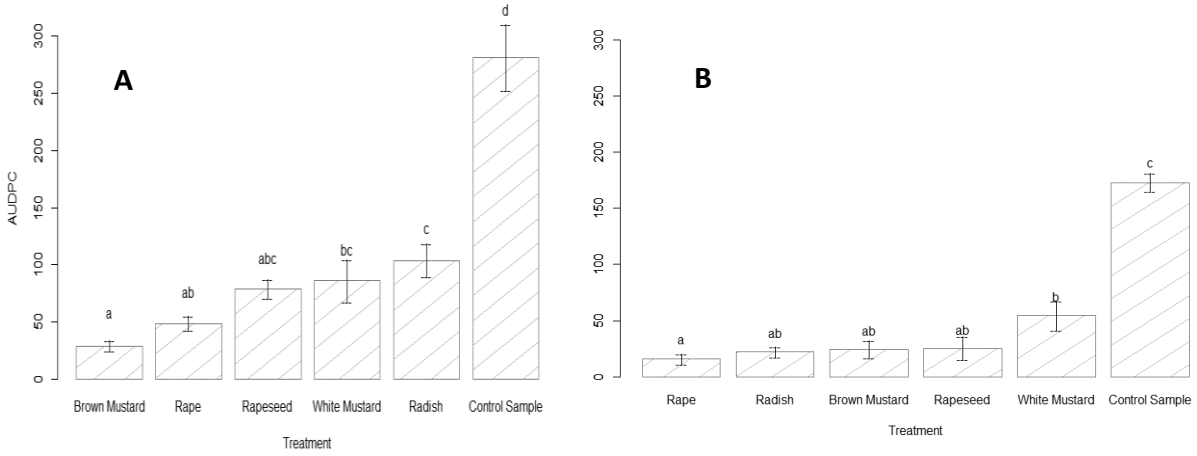


**Figure 1.** Effect of cover crop types and non-amended control on *V. dahliae* development and germination as measured by the area under the fungus development progress curve (AUDPC) for pooled data from conditioning and type of biomass from the fungus developed (DV) (**A**) and the plug of microsclerotia (MSV) (**B**). Within figures, means followed by letters are significantly different from one another based on LSD ($P < 0.05$).

## DISCUSSION

The aim of using cover crop to manage soilborne diseases is mainly the elimination of the inoculum source to reduce yield losses caused by pathogens infections. In this study, assessing the potential of *Brassica* for their biofumigation potential toward *V. dahliae* mycelial growth and the ability of microsclerotia to germinate was evaluated *in vitro*. Optimizing this method for control of *V. dahliae* requires knowledges of the type and concentration of the different GSLs occurring in the respective plant tissues. Therefore, the relative toxicity of ITCs released by their precursor GSLs in root and shoot biomass of white and brown mustard, radish, rape and rapeseed toward *V. dahliae* was tested.

In this study, the five cover crop treatments resulted in statistically significant ($P < 0.05$) reduction of the fungus development from *V. dahliae* developed (DV) and from microsclerotia (MSV) compared with the non-amended control. The toxicity of biological

compounds induced by the grinded biomass and more specifically the ITC-liberating GSLs in the tissues of *Brassica* crops towards *V. dahliae* was confirmed, which is in accordance with other studies testing *in vitro* toxicity of ITCs to other soilborne fungi (Manici et al., 1997 ; Sarwar et al., 1998 ; Smith and Kirkegaard, 2002,). However, the toxicity of ITC showed contrasted effect depending on whether the fungus was mycelial developed or from microsclerotia, which has not been investigated in the literature before. From DV, the mycelial growth of *V. dahliae* was significantly reduced with brown mustard and rape. From MSV, rape, brown mustard and radish blocked the germination of MS.

Brown mustard produced amounts of 2-propenyl ITC from sinigrin GSL (Kirkegaard and Sarwar, 1998; Morra and Kirkegaard, 2002) and the toxicity of this aliphatic GSL could have a significant biofumigation potential as confirmed towards *V. dahliae* and other soilborne pathogens (Angus et al., 1994; Mayton et al., 1996 ; Olivier et al., 1999 ; Smolinska and Horbowicz, 1999 ; Larkin and Griffin, 2007 ; Neubauer et al., 2014). For the DV treatment, the radish could be rated as a poor biofumigation crops as concluded by Neubauer et al., (2014) who evaluated the biofumigation potential of the culture by the aliphatic and aromatic GSL concentrations. However, the radish blocked significantly the germination of MS, more than the brown mustard. The high concentration of unknown indole 16.3 (indole GSL) could be involved but the biofumigant potential of indole GSL has not been studied against soilborne pathogens but more toward plant-parasitic nematodes (Ruanpanun et al., 2010 ; Kruger et al., 2013). Contrary to the radish and brown mustard, the sensitivity of the fungus toward rape biomass was equivalent in DV and MSV who reduced the viable MS by 90 % and *V. dahliae* growth by 83 % compared with the control. Despite the total concentration of aliphatic GSL (10.2 $\mu mol.g^{-1}$ DW) and indole GSL (10.9 $\mu mol.g^{-1}$ DW) was significantly lower than brown mustard (45.6 $\mu mol.g^{-1}$ DW) and radish (54.8 $\mu mol.g^{-1}$ DW) respectively, the rape biomass released a wider diversity of GSLs with aliphatic, aromatic and indole GSLs whereas those were specifics in one type of GSL. This could thus be involved in the high biofumigant potential of the rape, and the high concentration of one type of GSLs would not be predominant to reduce or suppress the development of *V. dahliae*. Regarding the incidence of white mustard and rapeseed to reduce the pathogen growth compared to the other, their profile predominant in gluconasturtiin and sinalbin (aromatic) GSL could explained this lower toxicity due to their lower volatility (Sarward et al., 1998). Although their biofumigation potential has been demonstrated before, aromatic GSLs released from these *Brassica* crops did not seems as effective for biofumigation as those from brown mustard, rape and radish.

## CONCLUSION

These results demonstrate that *Brassica* cover crop are able to reduce *V. dahliae* growth and microsclerotia germination. Moreover, the importance of identifying which GSLs type release the most toxic hydrolysis products toward the pathogen was underline. Inhibition of fungi development by grinded cover crops containing sinigrin (aliphatic), unknown indole 16.3 (indole) or a wider diversity of GSLs hydrolysis in *Brassica* tissue was superior to aromatic GSL, suggesting an important role for these compounds in the pest suppression potential of some *Brassica*. The variation in toxicity of different GSLs - ITCs to the fungi suggests there is significant scope to enhance the biofumigation potential of these crops by selecting those which produce a wider diversity of GSLs precursors to the most toxic ITCs such as rape to suppress Verticillium wilt in sunflower fields.

## ACKNOWLEDGMENT

We thanks D. Raffaillac, M. Labarrere and P. Perrin for technical assistance in management of the greenhouse experiment. G. Van Franck, G. Rametti, F. Perdrieux and S. Giuliano are

gratefully acknowledge for the field experimentation with *Brassica* cover crops. GSL analyses were performed by B-Ware laboratory (Nijmegen, Netherlands)

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# STUDY OF THE GENOMIC DIVERSITY OF VERTICILLIUM SP. CAPABLE OF COLONIZING SUNFLOWER. HOW KNOWLEDGE OF PATHOGEN GENETIC STRUCTURE CAN BE COMBINED WITH CLASSICAL BREEDING APPROACHES TO GUIDE IT

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## ABSTRACT

Plant disease management approaches are mainly represented by resistance genes and agrochemicals that are used repeatedly until their efficacy is overcome by the targeted pathogen. Despite no sexual cycle observed, comparative genomics show extensive chromosomal rearrangements and lineage-specific genomic regions that increase *V. dahliae* evolutionary potential. Yet, the complex relationship between spatial pattern of disease, plant genetics, crop practices and the evolutionary dynamics of *Verticillium* population remains poorly studied. The objective of this study was to investigate how uniform is the genetic make-up of the pathogen presents within a field. A spatial analysis was performed on a field with sunflower disease history to generate regions of high or low disease prevalence. Two sunflower genotypes were sampled 45 times in predefined areas according to the disease prevalence map previously established: i) a Symptomatic (S) and ii) an Asymptomatic (AS). Qualitative PCR were carried out to: i) point out the presence of *Verticillium* in stems (VdFE1/VdFE2 primers pair), and ii) to determine the defoliating and race profiles of the strains studied (Ave1F/Ave1R and D_NDf/D_NDr primers pairs used as markers for increased virulence and defined on tomato and cotton). Results showed i) a full colonization of S genotype by *Verticillium dahliae* and 75% of AS genotype colonized by undetermined *Verticillium* sp.; and ii) the exclusive presence of *Verticillium dahliae* strains that do not carry Ave1 gene. Deeper investigation of genomic diversity of *Verticillium dahliae* will be presented to better determine *V. dahliae* strains profile capable of colonizing sunflower.

**Key Words :** Verticillium, host-pathogen interaction, agrosystem, disease prevalence, cultivar specificity

# EVALUATION OF SUNFLOWER (HELIANTHUS ANNUUS L.) HYBRIDS FOR PHOTOTHERMAL UNITS ACCUMULATION, OIL YIELD, OIL QUALITY AND YIELD TRAITS UNDER SPRING PLANTING CONDITIONS OF HARIPUR, PAKISTAN

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## ABSTRACT

Field experiment was conducted at University of Haripur, Pakistan during spring 2014, to explore the role of photothermal units on oil contents, fatty acids profile, yield and yield traits of four sunflower hybrids viz SMH-0917, NKS-278, SMH-0907 and Hysun-33. Sunflower hybrids were sown in spring and arranged under randomized complete block design with 3 replications under field conditions. Significant variation ($p<0.05\%$) was found among the sunflower hybrids for photothermal units requirements for flower initiation, flower completion and physiological maturity. Highest photothermal unit accumulation was found in Hysun-33 followed by SMH-0917 and SMH-0907. Highest seed oil contents and oil quality (highest linoleic acid and oleic acid percentage while least percentage of palmitic acid) was recorded in Hysun-33, SMH-0917 and SMH-0907. Overall Hysun-33, SMH-0917 and SMH-0907 performed better for head diameter, number of achenes per head, total dry matter yield (kg ha-1) and economic yield (kg ha-1) under field conditions. It was also inferred that the temperature and moisture availability positively influence the oil quality of sunflower hybrids under spring planting conditions. Variability found among the tested sunflower hybrids for photothermal units accumulation, oil content, oil quality and yield traits could be exploited in the breeding program for development of early maturing and high yielding local sunflower hybrids.

**Key Words :** Sunflower, photothermal units, oil content, fatty acid profile, yield and yield traits

# DETERMINING NEW AGGRESSIVE BROOMRAPE INFESTATION IN MEDITERRANEAN REGION OF TURKEY

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## ABSTRACT

*Orobanche* L. is a large genus mainly distributed throughout subtropical and temperate regions of the northern hemisphere. The Mediterranean region is one of the most important centers of diversity. The genus Orobanche has been represented by 39 species due to this new record in Turkey. (Zare et al., 2009) Sunflower cultivation has gradually increased in the eastern Mediterranean region since 2004. ). In 2011, sunflower broomrape began to appear in cultivated area in Adana and increased rapidly until today. Based on this research results and natural condition observations show that infested new broomrape races areas are increasing seriously year by year in the mediterranean region. As a result it will be required of new sources genetic resistance to the most virulent races or herbicide resistant hybrids for this region.

## INTRODUCTION

*Orobanche* L. is a large genus mainly distributed throughout subtropical and temperate regions of the northern hemisphere. The Mediterranean region is one of the most important centers of diversity. The genus Orobanche has been represented by 39 species due to this new record in Turkey. (Zare et al., 2009)

Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm, totally devoid of chlorophyll, that infects the roots of sunflower (*Helianthus annuus* L.) plants, drawing water and nutrients from them. This parasitic plant is regarded as one of the most important constraints on sunflower production in areas of eastern and southern Europe, the Middle East, Russia, Ukraine and China (Parker,1994). According to Kaya *et al.* (2004), about 80% of sunflower areas in Turkey (Thrace region) are infested with the seeds of the parasite. According to these authors, epiphytotic occurrence of broomrape is registered in this region each 20 years. Furthermore, the parasite forms new, more virulent races which overcome the resistance of the varieties and hybrids commonly used in production (Kaya *et al*., 2004; Pacureanu-Joita *et al*., 1998; Alonso, 1996; Fernandez-Martinez *et al*., 2000). This impedes effective control of broomrape.

Sunflower cultivation has gradually increased in the eastern Mediterranean region since 2004. In 2005, sunflower acreage and production in the region were tripled compared with 2004. There has not been any record on broomrapes in sunflower fields in eastern Mediterranean region yet, but broomrapes are considered a possible threat for sunflower fields in this area. Orobanche cernua Loef. causes considerable damage in sunflower fields in other regions of Turkey where sunflower has been sown for years and it may spread from those regions to the eastern Mediterranean region (Bülbül et al. (2009). In 2011, sunflower broomrape began to appear in cultivated area in Adana and increased rapidly until today ( Figure 1, 3) .

## MATERIALS AND METOD

Sunflower hybrids in the official registration trials which commercial sunflower hybrids belong private companies were tested against to new broomrape races in natural conditions between 2013-2014.

Broomrape observations were evaluated as Frequency (F) Intensity (I) and Attacking Rate (AR) based on Pustovoit method. The plants were accepted as resistant having % 0-10 Frequency and 0-1 AR values and (Vranceanu *et al.*, 1980). The plants had % 10-20 frequency as accepted tolerant .

F = % The number of plant with orobanche (The plant number infested

orobanche / Total plants in the row x 100)

I = The number of orobanche in one infested plant (Total orobanche /

Total plants infested orobanche in the row).

AR = F x I / 100 = The number of orobanche in one plant in the row.

Figure 1. Broomraper in Adana Region in Turkey

## RESULTS

High percentages of broomrape attack were registered in southeastern regions of Turkey during 2013 and 2014 growing seasons. According to 2013 observations, only sensitive varieties showed infection of broomrape (Table 1). In 2014 all plants in the set which contain official checks were susceptible or highly influenced and then We concluded that they could be new races (Table 2, Figure 2).

| Table1. Broomrape observations in natural conditions | | | | |
|---|---|---|---|---|
| | | 2013 | | |
| | | Adana (Ceyhan) | | |
| | | F | I | SD |
| No | Varieties | % | (piece) | (piece) |
| 1 | Candidate 1 | 0.3 | 3.0 | 0.01 |
| 2 | Candidate 2 | 7.9 | 3.42 | 0.27 |
| 3 | Candidate 3 | 9.4 | 2.08 | 0.2 |
| 4 | Candidate 4 | 9.4 | 2.43 | 0.23 |
| 5 | Candidate 5 | 10.1 | 4.98 | 0.5 |
| 6 | Candidate 6 | 11.1 | 3.66 | 0.41 |
| 7 | Candidate 7 | 14.6 | 4.55 | 0.66 |
| 8 | Candidate 8 | 15.8 | 2.94 | 0.46 |
| 9 | Candidate 9 | 16.1 | 3.43 | 0.55 |
| 10 | Candidate 10 | 17.1 | 2.3 | 0.39 |
| 11 | Candidate 11 | 19.8 | 2.27 | 0.45 |
| 12 | Candidate 12 | 21.2 | 3.17 | 0.67 |
| 13 | Candidate 13 | 25.8 | 2.47 | 0.64 |
| 14 | Candidate 14 | 29.2 | 7.47 | 2.18 |
| 15 | Candidate 15 | 29.6 | 3.02 | 0.89 |
| 16 | Candidate 16 | 30.8 | 4.53 | 1.4 |
| 17 | Candidate 17 | 45.5 | 2.46 | 1.12 |
| 18 | Candidate 18 | 57.9 | 3.1 | 1.79 |
| 19 | Candidate 19 | 100 | 8.91 | 8.91 |
| 20 | Candidate 20 | 100 | 10.23 | 10.23 |
| 21 | Candidate 21 | 100 | 6.66 | 6.66 |
| 22 | Candidate 22 | 100 | 8.45 | 8.45 |
| 23 | Check1 | 1.5 | 3.33 | 0.05 |
| 24 | Check2 | 1.8 | 1.86 | 0.03 |
| 25 | Check3 | 2.8 | 3.73 | 0.1 |
| 26 | Check4 | 11.0 | 2.0 | 0.22 |
| 27 | Check5 | 24.8 | 2.4 | 0.6 |
| 28 | Check6 | 35.4 | 3.64 | 1.29 |

| Table2. Broomrape observations in natural conditions | | | | |
|---|---|---|---|---|
| | | 2014 | | |
| | | Adana (Sarıçam) | | |
| | | F | I | SD |
| No | Varieties | % | (piece) | (piece) |
| 1 | Candidate 1 | 18.0 | 1.35 | 0.24 |
| 2 | Candidate 2 | 100 | 6.72 | 6.72 |
| 3 | Candidate 3 | 78.4 | 1.22 | 0.96 |
| 4 | Candidate 4 | 84.4 | 1.42 | 1.2 |
| 5 | Candidate 5 | 100 | 2.11 | 2.11 |
| 6 | Candidate 6 | 80.3 | 1.99 | 1.6 |
| 7 | Candidate 7 | 100 | 12.43 | 12.43 |
| 8 | Candidate 8 | 100 | 11.69 | 11.69 |
| 9 | Candidate 9 | 100 | 8.11 | 8.11 |
| 10 | Candidate 10 | 86.8 | 2.57 | 2.23 |
| 11 | Candidate 11 | 100 | 3.91 | 3.91 |
| 12 | Candidate 12 | 86.8 | 6.94 | 6.02 |
| 13 | Candidate 13 | 100 | 6.25 | 6.25 |
| 14 | Candidate 14 | 100 | 4.12 | 4.12 |
| 15 | Candidate 15 | 100 | 11.98 | 11.98 |
| 16 | Candidate 16 | 100 | 15.8 | 15.8 |
| 17 | Candidate 17 | 100 | 8.08 | 8.08 |
| 18 | Candidate 18 | 90.2 | 4.38 | 3.95 |
| 19 | Candidate 19 | 100 | 10.86 | 10.86 |
| 20 | Candidate 20 | 98.5 | 12.19 | 12.01 |
| 21 | Candidate 21 | 100 | 11.55 | 11.55 |
| 22 | Candidate 22 | 100 | 15.33 | 15.33 |
| 23 | Check1 | 49.8 | 1.39 | 0.69 |
| 24 | Check2 | 100 | 1.52 | 1.52 |
| 25 | Check3 | 45.1 | 2.24 | 1.01 |
| 26 | Check4 | 100 | 10.41 | 10.41 |
| 27 | Check5 | 100 | 8.0 | 8.0 |
| 28 | Check6 | 100 | 2.84 | 2.84 |

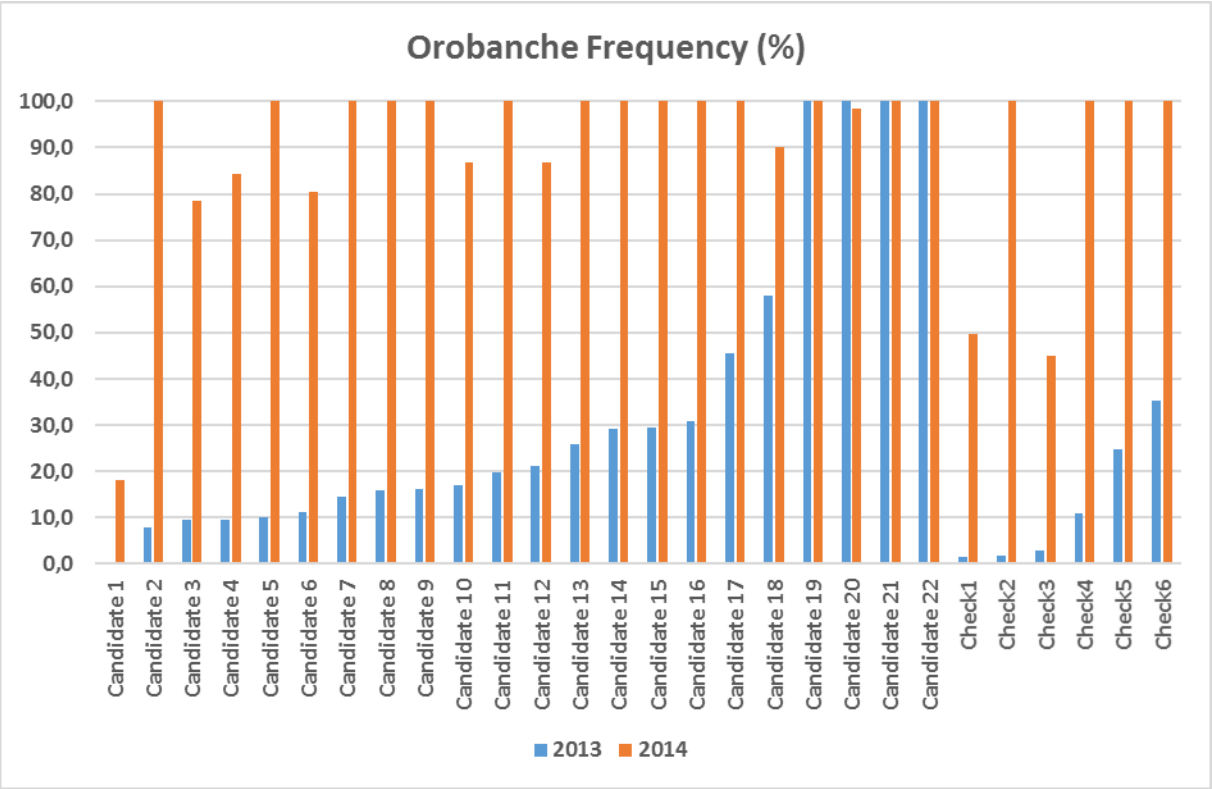


Figure 2: Broomrape density and frequency in trials

Figure 3: Broomrape in the fields.

## CONCLUSIONS

In recent years, the parasite *Orobanche sp.* has developed new and virulent populations, in the sunflower crop in Europe, including Turkey.

Based on this research results and natural condition observations show that infested new broomrape races areas are increasing seriously year by year in the mediterranean region.

Control of this parasite remains extremely difficult, as thousands of tiny seeds produced by one single broomrape plant can be easily dispersed by wind, water, animals, humans, machinery or attached to sunflower seeds. Broomrape seeds may remain viable for 15-20 years and will only germinate in the presence of the host plant (Škorić, 1988).

As a result it will be required of new sources genetic resistance to the most virulent races or herbicide resistant hybrids for this region.

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# STUDY OF *OROBANCHE CUMANA* GENETIC DIVERSITY

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## ABSTRACT

The study of *Orobanche cumana* genetic diversity is critical to better understand the evolution of this sunflower parasitic plant and develop new resistant sunflower hybrids. A broad collect of *Orobanche cumana* populations has been organised since 2012 in major countries affected by broomrape (Spain, Turkey, Romania, Hungary, Ukraine, Russia and France) resulting in the harvest of more than 500 orobanche seed lots. A subset of 12 populations representing different level of aggressiveness and different countries has been submitted to transcriptome sequencing in order to performed SNP discovery. This approach lead to the discovery of 368,000 SNP bi-allelic among which 1536 were selected for genotyping of the entire set of collected seed lots. This large diversity study outlined contrasted level of fixity between populations, more specifically French and Spain populations are largely homozygous compared to populations coming from Eastern Europe greatly heterozygous. The principal component analysis based on the relationship matrix (PCoA), allows the discrimination of population according to their geographical origin with 2 opposed clusters on axis 1 representing French and Spanish populations and a third cluster representing eastern Europe populations that can be split by countries on axis 3. Finally, this study allowed the definition of a diversity kit of 200 SNP that can be routinely used for *Orobanche Cumana* diversity study, this SNP toolkit is freely available upon demand to Biogemma.

**Key Words :** broomrape, genetic diversity, SNP

The authors acknowledge the financial support of SOFIPROTEOL under the FSRSO Project DIVO.

# REACTION OF SUNFLOWER (HELIANTHUS ANNUUS L.) LINES TO DROUGHT STRESS BASED ON TOLERANCE INDICES

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## ABSTRACT

In order to evaluation of genetic diversity of oily sunflower lines, screening drought resistance indices and identification of drought resistance lines, 100 lines of oily sunflower were evaluated in a simple lattice design with two replications under two conditions including normal irrigated and drought stress under filed condition in Salmas. Based on the potential (Yp) and stress (Ys) yield, quantitative drought tolerance criteria such as: mean productivity (MP), tolerance index (TOL), geometric mean productivity (GMP), harmonic mean (HM), stress susceptibility index (SSI) and stress tolerance index (STI) were calculated. Generally in both condition, line with code number of 8 with average yield of 81.25 g m-2 and line 66 with average yield of 5.425 g m-2 had the maximum and minimum values of yield. In normal and drought stress conditions, the highest value of MP, GMP and HM were possessed to genotype 8. Correlation analysis between drought resistance indices with potential and stress yields revealed that indices including MP, GMP, HM and STI are most suitable criteria for screening sunflower's genotypes. Line 8 was chosen as best drought resistant regarding to these four criteria and high values of Yp and Ys.

**Key Words :** Multivariate Analysis, Sunflower, Tolerance Index, Yield, Water Deficit

# CADMIUM-POTASSIUM INTERRELATIONSHIPS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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## ABSTRACT

Cadmium (Cd) is a toxic heavy metal for all living organisms. In this study, tolerance and bioaccumulation of Cd and mitigation of its toxic effects with potassium (K) treatments in sunflower (*Helianthus annuus* L., cv. Sirena) were investigated. Five levels of Cd (0, 0.1, 0.3, 0.6 and 1.2 mM) and three levels of K (0, 200, 400 mg kg-1) applied to the soil. Increasing Cd levels depressed root length and shoots and roots dry weight (DW), total chlorophyll and carotenoid, resulting from its toxic effects. However, these decreases were slightly ameliorated by applied K. Increasing Cd levels significantly increased membrane permeability (MP). Also, the shoot and root Cd contents, uptakes and total accumulation rate (TAR) of sunflower plant were increased by Cd treatments. These parameters all showed a declining trend with applied K. Moreover, shoot and root K content and uptake of sunflower increased considerably with applied K. The shoot and root bioconcentration factor (BCF) and translocation factor (TF) of Cd were decreased by applied K.

**Key Words :** Cadmium toxicity, growth, bioaccumulation, translocation, Helianthus annuus L., potassium

# RESPONSE TO SUNFLOWER (*HELIANTHUS ANNUUS* L.) PLANT AT EARLY GROWTH STAGE TO CADMIUM TOXICITY

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Cadmium (Cd) which is a non-essential element for plants, animals and humans has been a major pollutant in both terrestrial and aquatic environments for several decades. The effect of Cd toxicity was studied in sunflower (*Helianthus annuus* L., cv. Sirena) grown in greenhouse under natural light conditions. For this reason, the soil was treated with six levels of Cd (0, 0.05, 0.10, 0.25, 0.50 and 1.00 mM). Plant growth, photosynthetic pigments, relatively water content (RWC), bioaccumulation and translocation of Cd and uptake of zinc (Zn), potassium (K), and calcium (Ca) were investigated. Shoot and root growth and root elongation were depressed with increasing Cd levels and deleterious effect of Cd on plant growth was appeared shoot more than roots. Also, the contents of chlorophyll (Chl) and carotenoid (Car), RWC, growth tolerance index (GTI), uptake of Zn, K, and Ca in shoot and root were decreased with Cd application as well as bioaccumulation and translocation of Cd. Moreover, Cd treatments increased Cd content and uptake in shoot and root, total accumulation rate (TAR) of Cd, membrane permeability (MP), rate of Car/Chl caused by its toxic effects.

**Key Words :** Cadmium, toxicity, translocation, accumulation, growth, Helianthus annuus L.

# THE VIRULENCE OF PLASMOPARA HALSTEDII IN THE SOUTHERN REGIONS OF RUSSIAN FEDERATION

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## ABSTRACT

The population of oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni (sunflower downy mildew causal agent) has been monitored in Krasnodar Krai, Rostov region and Republic of Adygea more than 15 years. Prior to the beginning of the 2000s there were races 100, 300, 310 and 330 in the region. In the period from 2004 to 2007 races 100, 300, 310 and 700 met sporadically. The race 330 was the most common; in a number of agrocoenoses it was 100 % of samples. In some fields races 710 and 730 prevailed. In 2008-2011 only races 330, 710 and 730 were found; the race 330 still prevailed and was also found on *Ambrosia artemisiifolia* L. Since 2012 in the majority of fields races 710 and 730 prevailed, and the race 330 wasn't allocated in many of them; for the first time in Russia pathotype 334, that able to overcome $Pl_6$, was found in Krasnodar Krai. In the period of 2013-2015 increased distribution of the race 334 in Krasnodar Krai and Republic of Adygea was observed. At the same time, in 2014 in one field in the Rostov region only races 310 and 330 (prevailed) were identified. The virulence of the pathogen population is closely connected with the cultivated assortment of sunflower. Further spread and accumulation of P. halstedii race 334 and the emergence of new pathogen pathotypes in the said regions are predicted.

**Key words:** downy mildew, *Plasmopara halstedii*, races, sunflower, *Helianthus annuus, Ambrosia artemisiifolia*

## INTRODUCTION

One of the most spread and harmful diseases of sunflower (*Helianthus annuus L.*) in Russia is downy mildew, caused by oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni. The population of the parasite has been studied in the south regions of Russian Federation (Krasnodar Krai, Rostov region and Republic of Adygea) more than 15 years.

Initially, in the territory of the former USSR there was only race 100. However, in the early 1980s all resistant sunflower varieties of domestic selection became affected in the Krasnodar region (Tikhonov, Zaychuk, 1984). Prior to the beginning of the 2000s there were identified races 100, 300, 310 and 330 in the region (Antonova, 2003). It should be noted that over the previous decade foreign sowing material of sunflower was freely delivered in the country and decline in terms of return of the culture in field has become the norm.

In the period from 2004 to 2007 seven races of *P. halstedii* were found in the said regions. The most common was the race 330; in several agrocoenoses it was 100 % of samples. Races 710 and 730 prevailed in some fields. Races 100, 300, 310 and 700 met

sporadically. Status of the pathogen population in the region in those years was described in detail previously (Antonova et al., 2008).

The aim of our study was to monitor the racial structure of *P. halstedii* population in the southern regions of Russian Federation (Krasnodar Krai, Rostov region and Republic of Adygea).

## MATERIALS AND METHODS

The leaves from the infected by downy mildew sunflower plants were collected from the fields in Adigeya republic, Krasnodar and Rostov areas in 2009-2015 (table 1).

Table 1. The total numbers of identified *P. halstedii* isolates and fields, where isolates of *P. halstedii* were collected, in different years

| Total number | Years | | |
|---|---|---|---|
| | 2009-2011 | 2012-2013 | 2014-2015 |
| identified *P. halstedii* isolates | 196 | 474 | 480 |
| surveyed fields | 11 | 16 | 19 |

For identification of pathogen races, according to the nomenclature system (Tourvieille et al., 2000), nine *P. halstedii* differential lines of *H. annuus* were used: set 1 – VNIIMK 8883 (D-1), RHA-265 (D-2), RHA-274 (D-3); set 2: DM-2 (D-4), PM-17 (D-5), 803-1(D-6); set 3: HA-R4 (D-7), HA-R5 (D-8), HA-335 (D-9). The line HA-304 (D1), that was not stable in reaction of resistance or susceptibility, has been changed on the universally susceptible variety VNIIMK 8883.

Pre-germinated seeds of differentials with radicle length 1,0-2,0 cm were placed in plastic growth trays with sterilized sand, covered by filter paper. The radicles of seedlings were covered by wet cotton wool. 150 ml of zoosporangial suspension of isolates (concentration about $10^6$ zoosporangia/ml) were added in growth trays (one *P. halstedii* isolate per tray) and incubated 16-20 hours at the temperature 16 $^oC$. Inoculated sunflower plants were grown at the temperature 25±2 $^oC$ (16 h photoperiod) and after 7-9 days were placed in darkness at 16 $^oC$ in 100 % humidity overnight for induction of *P. halstedii* sporulation. Plants with sporulation on leaves or with abundant sporulation on cotyledons only were classified as susceptible.

## RESULTS

Until 2007 in the southern regions of Russia (Krasnodar Krai, Rostov region and Republic of Adygea) seven races of *P. halstedii* were found. Among them during 2004-2007 races 100, 300, 310 and 700 constituted together about 2,5 %, and the most common were races 330, 710 and 730 (about 65, 13,5 and 19 % respectively).

Since then, there have been significant changes in the structure of the pathogen population. They are shown in table 2, which presents the prevalence and frequency of occurrence of races in the region in different years.

Table 2. The distribution of *Plasmopara halstedii* races on sunflower in southern regions of Russia during 2004-2015*

| Races | Years | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2004-2007 | | | 2009-2011 | | | 2012-2013 | | | 2014-2015 | | |
| | F | I | Ifi | F | I | Ifi | F | I | Ifi | F | I | Ifi |
| 100 | 0.05 | 0.2 | 3.3-7.1** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 300 | 0.07 | 0.7 | 1.2-3.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 310 | 1.6 | 0.9 | 1.2-10.3 | 0 | 0 | 0 | 0 | 0 | 0 | 5.3 | 1.8 | 17.2 |
| 330 | 100 | 65.1 | 12.7-100 | 100 | 46.5 | 25.0-92.0 | 75.0 | 18.5 | 7.7-25.6 | 31.6 | 26.2 | 10-91.4 |
| 700 | 1.3 | 0.7 | 1.2-12.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 710 | 70 | 13.6 | 2.3-69.6 | 100 | 25 | 3.3-57.1 | 100 | 35.7 | 26.6-90.0 | 73.7 | 24.0 | 5.0-54.0 |
| 730 | 56 | 18.8 | 3.3-58.2 | 100 | 28.5 | 6.7-52.1 | 93.8 | 44.5 | 16.7-70.2 | 73.7 | 30.5 | 10.0-64.0 |
| 334 | 0 | 0 | 0 | 0 | 0 | 0 | 12.5 | 1.3 | 0.4; 2.2 | 47.4 | 17.5 | 20.0-100 |

* F - the frequency of the race occurrence in the fields, %;

I - race proportion in the total number of identified *P. halstedii* isolates, %;

Ifi - minimum and maximum percents (%) of the race in positive samples, %

**- the samples of *P. halstedii* isolates were small

Race 100, 300 and 700 were not found after 2007. However oospores of *P. halstedii* are capable of being viable in the soil up to 10 years (Viranyi and Spring, 2011). Therefore it is not excluded that these races still are present in the region as another one of the old races - 310, which was found in one field in 2014 and amounted to 17 % of the sample (table 2). The period of existence of these races in the pathogen population prolongs by cultivation of susceptible sunflower in separate fields.

Till 2011 race 330 was found in each of surveyed fields and dominated in the south of Russia. But from 2012 its part in the pathogen population has considerably decreased: it became less than 20 % in 2012-2013 and less than 30 % in 2014-2015. In 2012-2013, it was present in 75 % of samples, in 2014-2015 – only in 32 %. At the same time, race 330 has been found on plants of common ragweed (*Ambrosia artemisifolia* L.) in Krasnodar Krai in different years (2011, 2013 and 2015). All isolates collected by us from ambrosia belonged only to this race. Analyze of SNP DNK locuses proved identity of this isolates and isolates of the race 330 from sunflower (Iwebor et al., 2012). Thus the race 330 can persist in local population of *P. halstedii* on ambrosia. Even the widespread cultivation of sunflower, resistant to this race, will not lead to its complete disappearance, as in Russia *A. artemisiifolia* is ubiquitous in areas of sunflower cultivation.

Races 710 and 730 were found only in 70 and 56 % (respectively) of the surveyed fields in 2004-2007 and they were found almost in every field in 2009-2013. Since 2012 these two races (individually or together) prevailed over race 330 in pathogen population both in general and in the majority of separate agrocoenosises.

In 2012 one isolate of race 334 was discovered in Krasnodar Krai. In Russia it was the first time of detection of the pathotype that able to overcome the resistance gene $Pl_6$ of sunflower. In 2013 the race 334 has been found again in one field. In 2014-2015 increased distribution of this race was observed in Krasnodar Krai and in Republic of Adygea (tables 3 and 4). It was present in almost half of surveyed fields and reached 17,5 % of the total number of identified pathogen isolates. Race 334 ranged from 20 to 100 % in the samples from different field (table 2).

All changes which have happened in racial structure of *P. halstedii* population were closely connected with cultivated assortment of sunflower that was clearly demonstrated in the tables 3 and 4.

Table 3. Races of *P. halstedii*, found in the sunflower fields in the Republic of Adygea and Rostov region in 2011-2015

| Location of the field | Year | Foreign hybrids of sunflower in the field* | The number of isolates | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | total | race | | | | |
| | | | | 310 | 330 | 710 | 730 | **334** |
| Rostov region | 2011 | - | 19 | 0 | 6 | 6 | 7 | 0 |
| | 2014 | - | 55 | 0 | 40 | 7 | 8 | 0 |
| | | - | 64 | 11 | 53 | 0 | 0 | 0 |
| Republic of Adygea | 2012 | - | 28 | 0 | 7 | 11 | 10 | 0 |
| | 2014 | + | 10 | 0 | 0 | 5 | 2 | **3** |
| | 2015 | + | 16 | 0 | 0 | 3 | 3 | **10** |

* - foreign hybrids of sunflower have been cultivated in the field in any of last 5 years (before the year of sampling): '+' – yes, '-' – no

In one of the sunflower fields in the Rostov region (2014), race 330 dominated and race 310 has been found. Races 710 and 730 have not been revealed there (table 3). From the history of the field it is known that only domestic sunflower varieties were cultivated there. In the other two fields of this region and in one of the fields in Adygea (2012) also domestic varieties and hybrids were grown only. There were identified races 330, 710 and 730. Race 334 was found in the Republic of Adygea in two fields, in which during several last rotations of sunflower foreign hybrids were cultivated.

The similar situation was observed in fields of Krasnodar Krai (table 4). In the fields, where only domestic varieties and hybrids have been cultivated (at least 5 last years before the year of sampling), races 330, 710 and 730 were isolated, but not the race 334.

Race 334 was revealed in the fields where in any of last 5 years (before the year of sampling) foreign hybrids of sunflower have been cultivated. It was such in the fields in Korenovsky and Tbilissky districts (Krasnodar Krai), where over the last 5 years foreign hybrids were sowed twice. In several samples race 334 made 100 %: these *P. halstedii* isolates were collected from the foreign sunflower hybrids with $Pl_6$ – the gene of resistance to all parasite landraces except 334.

Table 4. Races of *P. halstedii*, found in the sunflower fields in the Krasnodar Krai in 2011-2015

| Districts | Year | Foreign hybrids of sunflower in the field* | The number of isolates | | | | |
|---|---|---|---|---|---|---|---|
| | | | total | race | | | |
| | | | | 330 | 710 | 730 | **334** |
| Belorechensky | 2011 | - | 11 | 7 | 2 | 2 | 0 |
| Gulkevichsky | 2011 | - | 28 | 10 | 9 | 9 | 0 |
| | 2015 | - | 64 | 5 | 18 | 41 | 0 |
| Novokubansky | 2012 | - | 39 | 1 | 26 | 12 | 0 |
| Labinsky | | - | 12 | 0 | 9 | 3 | 0 |
| Kushchyovsky | | + | 3 | 0 | 2 | 0 | 1 |
| Korenovsky | 2013 | + | 50 | 24 | 2 | 40 | 34 |
| Tbilissky | 2014 | + | 70 | 11 | 5 | 5 | 49 |
| Slavyansky | 2015 | - | 12 | 7 | 4 | 1 | **0** |
| Novopokrovsky | | +** | 15 | 0 | 0 | 0 | 15** |
| | | - | 7 | 0 | 1 | 6 | **0** |
| Kanevskoy | | +** | 17 | 0 | 0 | 0 | 17** |
| Pavlovsky | | +** | 25 | 0 | 0 | 0 | 25** |

* - foreign hybrids of sunflower have been cultivated in the field in any of last 5 years (before the year of sampling): '+' – yes, '-' – no; ** - *P. halstedii* isolates were collected from the foreign hybrids with $Pl_6$

Russia became the second European country in which race 334 has been revealed. This race was registered for the first time at the beginning of the 2000 in France and after 2007 - in the USA and Canada (Gulya,2007; Delmotte et al., 2008; Viranyi et al., 2015).

It is possible that race 334 was introduced into our country with the seeds of foreign sunflower hybrids. On the other hand, its appearance could become the result of evolutionary processes in local *P. halstedii* population, exerted by cultivating of resistant sunflower hybrids and elevated by crop rotation violations.

Experience of different countries showed that after the appearance of new races in the population of this parasite, the emergence of other races can be expected soon. As it was happened in France. After the race 100, there emerged pathotypes with virulence code 7xx which have overcome the resistance of differential lines RHA-274 (D-3): races 710 and 703 to which also lines PMI3/DM-2 (D-4) and HA-R4 (D-7) + HA-R5 (D-8) (respectively) are susceptible. Then, due to the massive deployment of new resistance genes (as $Pl_6$ and $Pl_7$), there were formed new races, that able to overcome resistance of differential lines RHA-265 (D-2) – races 3*xx*, PM-17 (D-5) – races *x*3*x*, HA-335 (D-9) – races *xx*4 и *xx*7 (Tourvieille de Labrouhe et al., 2005; Delmotte et al., 2008; Viranyi et al., 2015).

In the south of Russia after race 100, races with virulence code 3x0 (300, 310 and 330) appeared, then – 7x0 (700, 710 and 730) and the last to date – race 334. The pathotypes with

virulence to the genes of resistance in differential lines 803-1 (D-6), HA-R4 (D-7) and HA-R5 (D-8) still were not recorded here.

Thus, the racial composition of the *P. halstedii* population in the south of Russian Federation has changed due to the cultivated assortment of sunflower. Races 330, 710 and 730, which dominated last years, still were widespread but their parts in the parasite population decrease. In 2012, for the first time in Russia, the race 334 has been found. It is quickly distributes and has occupied a dominant position in some fields. Further spread and accumulation of *P. halstedii* race 334 and the emergence of new pathogen pathotypes in the southern regions of Russia are predicted.

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# QUANTIFICATION OF DROUGHT TOLERANCE LEVELS OF SUNFLOWER INBRED LINES BY MEANS OF CHLOROPHYLL-*A* FLUORESCENCE

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## ABSTRACT

Plants are often exposed to various environmental stresses such as drought. One of the major objectives in plant breeding programs for crops grown in arid/semiarid areas is selection of crop cultivars with remarkable resistance to drought stress. To select drought tolerant lines, chlorophyll *a* fluorescence (ChlF) measurements has used in addition to morphological and physiological analysis in recent years. Some sunflower (*Helianthus annuus* L.) inbred lines developed by Trakya Agriculture Research Institute (TARI) with National Sunflower Project were grown in Bahri Dagdas International Agricultural Research Institution in order to determine the drought tolerance levels using fast ChlF techniques. Fluorescence signals were recorded and analyzed using JIP-test. $V_J$, $V_I$, ABS/RC, $ET_0/TR_0$, $DI_0/RC$, $RE_0/ET_0$ and $PI_{total}$ originated from JIP-test parameters were evaluated. Besides, drought factor index (DFI) was calculated using data of $PI_{total}$ and lines were classified according to their drought tolerance levels. Results obtained from present study indicated that lines were markedly affected depending on the duration and severity of the drought. Additionally, sunflower inbred lines could be separated into four groups as highly drought tolerant (9814 A), drought tolerant (TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A) less drought tolerant (8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107 A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A) and drought sensitive (6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A) based on the DFI values.

**Key words:** Sunflower, Drought tolerance, Inbred lines, Chlorophyll *a* fluorescence kinetics

## INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important agricultural crops in the world and the main source of unsaturated vegetable oil (Baloğlu et al., 2012; Gholinezhad et al., 2015). While the world sunflower production has varied from 2010, production of Turkey has been increased (FAOSTAT, 2016). Turkey has among the top 10 in sunflower producer countries according to National Sunflower Association data. On the contrary of well-studied model plants, different water regimes adaptable plants could be valuable genetic sources to understand links between stresses and stress responses (Raineri et al., 2015). Due

to its drought tolerance and deep root system compared to other crops, sunflower has been an attractive alternative genetic source (Howell et al., 2015).

Among the various abiotic stresses, drought is the most significant environmental stress in agriculture worldwide and under the limited water conditions improving yield and yield capacity is major goal of plant breeder. Due to urbanisation, industrialisation, depletion of ground-water and global warming, the amount of available water is decresing day by day. dorught triggered by these conditions cause major constraints on the physiology, biochemistry, growth, development and productivity of plants (Bechtold et al., 2016; Szechyńska-Hebda et al., 2016) depending on the stres intensity and duration (Raineri et al., 2015). More than 80 years of breeding activities have caused some yield increase for crop plants grown in areas affected by drought (Cattivelli et al., 2008) and this situation would be the most economical approach to improving agricultural productivity and reducing agricultural use of substantial water resources (Sperdouli and Moustakas, 2012). Drought stress primarily influences photosynthesis, by multidimensional ways just as reduce in leaves expansion, decreased $CO_2$ diffusion to the chloroplast, impared photosynthetic apparatus with enzymes and expedite of leaf senecence (Farooq et al., 2009; Pinheiro and Chaves, 2011; Hasanuzzaman et al., 2014). Based upon limitation of $CO_2$ uptake and imbalance between absorbing and using of sunlight, the possibility of overexcitation of photosystem II (PSII) increases. This case induces a decrease of photosynthetic rate and an increase in the dissipation of absorbed energy through non-radiative processes (Faraloni et al., 2011). Under drought conditions, photosystem II (PSII) is more sensitive than photosystem I (PSI) (Deng et al., 2003), therefore PSII has a key role to analyze changes that occur in photosynthesis (Baker, 1991). ChlF is a non-invasive measurements of PSII activity and is a commonly used technique (Murchie and Lawson, 2013; Schansker et al. 2014). To determine the photosynthetic performance, ChlF kinetics can be considered as a biosensor tool. All oxygenic photosynthetic samples investigated so far using ChlF techniques show the characteristic polyphasic rise from the ground state value ($F_0$, 20μs) at the O step to its maximum value ($F_M$, approx. 300-500 ms) at the P step with J ($F_J$, 2 ms) and I ($F_I$, 30 ms) intermediate steps (Strasser et al. 2004). An analysis of the fast OJIP fluorescence kinetics, called JIP test, quantifies the in vivo energy fluxes passing through the reaction centres and photosystems (Strasser and Strasser, 1995; Strasser et al., 2000). An analysis of the fast OJIP fluorescence kinetics, called JIP test, links different steps and phases of the transient with the redox states of PSII, also correlates the phases with the efficiencies of electron transfer in the intersystem chain between PSII and PSI and to the end electron acceptors at the PSI acceptor side (Strasser et al., 2004).

The aim of this study was to evaluate the effects of drought on ChlF kinetics in sunflower female inbred lines developed in National Sunflower project (TÜBİTAK-113O926) conducted by TARI under controlled conditions in Konya, Turkey.

## MATERIALS AND METHODS

The study was conducted in Bahri Dagdas International Agricultural Research Institution's research fields in 2014. A total of fifty female inbred lines, originated different genetic sources, were initially sown, however the measurements of 38 lines were used to analyse the drought tolerance, and the rest of lines were excluded. Trials were conducted in controlled environmental conditions with randomized complete block design with one row and three replications. In each row, there were 5 plants and the distance between rows 70 cm and in rows were 30 cm. Trials were planted by hand in 31 May and drip irrigation was

applied and as covering rain shelters. Chlorophyll fluorescence measurements were three times like below in the experiments.

**Control:** All plant water need were supplied by drip irrigation.

**Stress group 1 ($S_1$):** 65-day-old sunflower lines under natural condition without irrigation (R3 stage).

**Stress group 2 ($S_2$):** 75-day-old sunflower lines under natural condition without irrigation (R5-1 stage).

**Stress group 3 ($S_3$):** 85-day-old sunflower lines under natural condition without irrigation (R6 stage).

At the end of the treatments, polyphasic ChlF measurements were carried out from leaves. The polyphasic OJIP fluorescence transient was measured with a Handy PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) fluorimeter. Samples dark-adapted for at least 30 min were illuminated with continuous light (650 nm peak wavelength, 3000 μmol $m^{-2}$ $s^{-1}$ maximum light intensity, for 1 s) provided by 3 LEDs, and the Chl a fluorescence signals were recorded according to Strasser and Strasser (1995). The OJIP transient was analysed by JIP test, based on the energy flux theory for biomembranes in a photosynthetic sample, which leads to the equations and calculations for the efficiencies of the whole energy cascade from absorption to the reduction of end electron acceptors at the PSI acceptor side and the performance indexes (Tsimilli-Michael et al., 2000; Strasser et al., 2004; Strasser et al., 2010). The fluorescence parameters (Table 1) were calculated using the Biolyzer software package.

Drought factor index (DFI) was calculated from using data of performance index ($PI_{total}$) and sunflower lines were ranked. DFI was calculated according to Strauss et al. (2006) and Oukarroum et al. (2007) with minor modification and calculated with the formula: DFI = $logA + 2\ logB + 4\ logC$, where C is the average relative performance index (PI) during the first treatment of drought, B is the average relative $PI_{total}$ during the second treatment, and A is the average relative $PI_{total}$ of the third treatment. The relative $PI_{total}$ was calculated as $PI_{drought}/PI_{control}$.

Experimental data were subjected to Analysis of Variance (ANOVA) using the statistical software SPSS Statistics. Means were compared with least significant differences (LSD) at 5% level ($P<0.05$).

## RESULTS AND DISCUSSION

Photosynthesis, the key process of plant metabolism, is strongly influenced by environmental conditions (Kalaji et al., 2014). Measurements of photosynthetic efficiencies are an important component of agricultural, environmental, and ecological studies. ChlF measurements represent a simple, non-destructive, inexpensive and rapid tool allowing scientists to get information on the photosynthetic process without destroying the tested samples. The ChlF parameters are potentially useful for screening genotypes for drought tolerance (Oukarroum et al., 2007; Strasser et al., 2010; Boureima et al., 2012; Çiçek et al., 2015; Kalaji et al., 2016).

National Sunflower Project was conducted by TARI in Edirne. Many inbred female lines and $F_1$ hybrids producted within this project and registered for Turkey. To determine of the level of drought tolerance these lines were grown under field conditions in Konya,

Turkey. In this study, the effects of different drought stress on photosynthetic efficiency were examined by using the changes in some chlorophyll a fluorescence parameters (JIP-test parameters), such as $V_J$, $V_I$, ABS/RC, $ET_0/TR_0$, $DI_0/RC$, $RE_0/ET_0$ and $PI_{total}$. The means of these parameters were calculated across each treatment of all the sunflower hybrids and the values of stress groups were normalized by the values of the control plants (control value: 1) for each hybrid. In general, the changes in these parameters were observed compared to their controls.

The relative variable fluorescence at the J-step ($V_J$) increased almost all stress duration compared to control (Figure 1). $V_J$ values of sunflower lines were prominently increased depending on stress duration. The highest increases were observed in 8959A, 9728A, 9907A (more than 50% or almost 60% increase) under severe drought stress (S3). It has been suggests that the fluorescence yield at the J-step is strongly determined by the redox state of the electron carriers in the electron transport chain (Haldimann and Strasser, 1999). Drought stress might blocked or inhibited the re-oxidation of the electron carriers in the sunflower lines.

The relative variable fluorescence at the I-step ($V_I$) was significantly increased compared to control for all stress treatments (Figure 2). Depending on the drought intensity, the highest increase was observed in 2478 A, 9728 A, 9412 A and 97181 A hybrids (44-46 %) for S3. It has been suggested that $V_I$ values is as an approximate estimation of the fraction of QB-non-reducing PS II (Hsu and Lee, 1991). Drought stress significantly increased the fraction of $Q_B$-non-reducing PSII centers in almost all inbred sunflower lines. Electron transfer between $Q_A^-$ and $Q_B$ does not function in the $Q_B$-non-reducing reaction centers (Cao and Govindjee, 1990). This situation might affect the electron transport towards PS I. Moreover, an increased $V_I$ was used as a probe for the inhibition of electron transport at the acceptor side of PSII under stress condition (Chen et al., 2004).

It has been suggested that Drought Factor Index (DFI) represents the relative drought induced changes of Performance Index (PI) during a freely time of drought stress (Kalaji et al., 2016). Sunflower inbred lines investigated in present study were ranked according to their DFI values given on Table 2. Drought-tolerant genotypes with the lowest reduction in the $PI_{total}$ under drought stress had the largest (less negative) DFI values. Based on the DFI values, thirty eight sunflower inbred lines could be separated into four groups: 9814 A is the first group (highly drought tolerant; DFI: -1.19), TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A are the second group (drought tolerant; DFI: -1.58 - -1.90), 8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107 A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A are the third group (less drought tolerant; DFI: -2.01 - -2.48) and 6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A are the fourth group (drought sensitive; DFI: -2.53 - -3.01).

DFI is calculated from PI values, so it is closely related to PI as seen above. At all stages of development $PI_{total}$ was decreased significantly compared to control and depending on the drought periods (S1, S2 and S3) the highest decreases were in 6626 A and 9942 A (approx.35%), 8435 A and 8543 A (approx. 55%), 8959 A, 6545 A and 9728 A (approx. 75%) respectively (Figure 3). The performance index ($PI_{total}$) are used to utilize the effects of the environmental constraints on the plant. It has been proved that these parameters are more sensitive to the environmental changes than other fluorescence parameters, such as $F_V/F_M$ and they correlates well with plant vitality (Oukarroum et al., 2007; Tsimilli-Michael and Strasser,

2008, Kalaji et al., 2016; Siddiqui et al., 2016). $PI_{total}$ is predicating the performance up to the PSI end electron acceptors.

ABS/RC is reciprocal of RC/ABS utilized to calculate PI. ABS/RC known as average antenna size, expresses the total absorption of PSII antenna chlorophylls divided by the number of active (in the sense of QA reducing) reaction centers (Strasser et al., 2000). ABS/RC parameter was decreased in S1 and S2 (except for 9942A, 6626 A and 8543A) stages, the parameter values was increased approximately half of the hybrids in the S3 stage and the other half was close to control value or higher than it (Figure 4). Under stress conditions ABS/RC was increased by the reason of inactivation of the PSII reaction centers (van Heerden et al., 2007; Mladenov et al., 2015).

Probability that a trapped exciton moves an electron into the electron transport chain beyond $QA^-$ ($\Psi_0$, $ET_0/TR_0$) was significantly decreased in all stress groups compared to controls (Figure 5). In relation to the stress duration, the highest decrease in the $\Psi_0$ was observed in S3 treatment. Also, increase in $V_I$ value supports results of $\Psi_0$ obtained from this study. Lower $\Psi_0$ values might exhibit that the activity of electron transport beyond $Q_A$ was considerably inhibited. Jiang et al. (2006) and Oukarroum et al. (2015) reported similar relationship between these parameters.

$RE_0/ET_0$, efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors ($\delta R_0$), was significantly affected from drought stress durations dependent manner compared to control (Figure 6). As for $ET_0/TR_0$ and $PI_{total}$, $RE_0/ET_0$ values of sunflower inbred lines were decreased depending on duration and the severity of drought stresses. Schansker et al. (2005) stated that a lower $RE_0/ET_0$ level indicated a decrease of a traffic jam of electrons at the acceptor side of PSI caused by an inactivation of ferredoxin-$NADP^+$-reductase.

Chlorophyll fluorescence (ChlF) transients allow the evaluation of the physiological condition of photosystem II (PSII) and energy fluxes of thylakoid membranes. It also gives information on the cooperation of photochemical and nonphotochemical reactions. $DI_0/RC$ which expresses the ratio of dissipation to the amount of active reaction center (Strasser et al., 2000), was decreased in S1 and S2 (except for 9942 A, 6626 A and 8543 A) stages. In addition, for 2453 A, 2517 A, 62001 A, 6545 A, 6626 A, 8435 A, 8454 A, 8543 A, 8959 A, 9209 A, 9444 A, 9907 A, 9942 A and CL078 A hybrids $DI_0/RC$ was significantly higher in drought treated leaves than in controls (Figure 7). Dissipation of light energy per active reaction centers ($DI_0/RC$) can be thought of as the absorption of photons in excess of what can be trapped by the reaction centers as heat (Mathur et al., 2011). Reduction in energy dissipation would explain increase in the fluorescence emission by the excited antenna chlorophyll *a* molecules before the migration of excitation to the reaction centers (Falqueto et al., 2012). $DI_0/RC$ might be increased to avoid photo-oxidative damage of photosynthetic apparatus.

## CONCLUSION

Drought stress affected adversely the photosynthetic efficiency of examined sunflower hybrids. The tolerance levels of sunflower inbred lines were determined using ChlF techniques. Sunflower lines could be classified into four groups as highly drought tolerant (9814 A), drought tolerant (TT 179 A, 0046 A, CL 068 A, 9725 A and 2517 A) less drought tolerant (8454 A, 9209 A, 9178 A, 9661 A, 8255 A, CL 078 A, 9444 A, TT 176 A, 96172107

A, TT 188 A, 2478 A, 9718 A, 6388 A, 9942 A and 62001 A) and drought sensitive (6626 A, 6163 A, 97181 A, TT 187 A, 9907 A, 7751 A, 917574 A, 6545 A, 9726 A, TT 198 A, 8428 A, 8435 A, 8543 A, 9412 A, 8959 A, 2453 A and 9728 A). The drought factor index calculated from PI parameter can be used to classify the level of stress tolerances. The use of supplementary parameters like PI can be more useful than complex biophysical parameters to understand the photochemical processes, also to interpret the data correctly.

***ACKNOWLEDGMENTS***

This study was *supported* by the Scientific and Technological Research Council of Turkey (*TÜBİTAK*) under the Project 1001- 113O926.

Table 1. ABSTRACT of the JIP test formulae using data extracted from the polyphasic chlorophyll a fluorescence (OJIP) transient in this study (Han et al., 2009; Strasser et al., 2010).

| **Data extracted from the recorded fluorescence transient OJIP** | |
|---|---|
| $F_0 = F_{20\mu s}$ | Initial fluorescence intensity, when all PSII RCs are open |
| $F_K = F_{300\mu s}$ | Fluorescence intensity at 300 ms |
| $F_J = F_{2ms}$ | Fluorescence intensity at the J-step (at 2 ms) |
| $F_I = F_{30ms}$ | Fluorescence intensity at the I-step (at 30 ms) |
| $F_M$ | Maximal fluorescence intensity, when all PSII RCs are closed |
| $V_J$ $(F_{2ms} – F_0)/(F_M – F_0)$ | Relative variable fluorescence at the J-step (2 ms) |
| $V_I$ $(F_{30ms} – F_0)/(F_M – F_0)$ | Relative variable fluorescence at the I-step (30 ms) |
| $M_0 = 4(F_{300\mu s} – F_0)/( F_M – F_0)$ | Approximated initial slope (in $ms^{-1}$) of the fluorescence transient normalized on the maximal variable fluorescence $F_V$ |
| ***Specific energy fluxes or activities expressed per reaction center (RC)*** | |
| $ABS/RC = M_0 (1/V_J)(1/\varphi_{Po})$ | Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size) |
| $DI_0/RC = ABS/RC - TR_0/RC$ | Dissipated energy flux per RC at t = 0 |
| ***Quantum yields and efficiencies/probabilities*** | |
| $F_V/F_M = \varphi_{Po} = TR_0/ABS = [1− (F_0/F_M)$ | Maximum quantum yield for primary photochemistry |

| | |
|---|---|
| $RC/ABS = \varphi_{Po} \times (V_J/M_0)$ | The concentration of reaction centres per chlorophyll |
| $\Psi_0 = ET_0/TR_0 = (1-V_J)$ | Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond $Q_A^-$ |
| $\delta R_0 = RE_0/ET_0 = (1 - V_I) / (1 - V_J)$, | Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors |
| ***Performance index (products of terms expressing partial potentials at steps of energy bifurcations)*** | |
| $PI_{total} = (RC/ABS) \times [\varphi_{Po} /(1 - \varphi_{Po})] \times [\Psi_0 /(1 - \Psi_0)] \times [\delta R_0/(1 - \delta R_0)]$ | Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors |

Table 2. Drought factor index (DFI) values of 38 sunflower inbred lines grown under drought stress. The inbred lines were ranked according to their DFI values.

| | **Lines** | **DFI Values** |
|---|---|---|
| 1 | 9814 A | -1,19 |
| 2 | TT 179 A | -1,58 |
| 3 | 0046 A | -1,80 |
| 4 | CL 068 A | -1,84 |
| 5 | 9725 A | -1,85 |
| 6 | 2517 A | -1,90 |
| 7 | 8454 A | -2,01 |
| 8 | 9209 A | -2,01 |
| 9 | 9178 A | -2,09 |
| 10 | 9661 A | -2,15 |
| 11 | 8255 A | -2,18 |
| 12 | CL 078 A | -2,23 |
| 13 | 9444 A | -2,26 |
| 14 | TT 176 A | -2,26 |
| 15 | 96172107 A | -2,31 |
| 16 | TT 188 A | -2,36 |
| 17 | 2478 A | -2,38 |
| 18 | 9718 A | -2,44 |
| 19 | 6388 A | -2,45 |
| 20 | 9942 A | -2,47 |
| 21 | 62001 A | -2,48 |
| 22 | 6626 A | -2,53 |
| 23 | 6163 A | -2,57 |
| 24 | 97181 A | -2,58 |
| 25 | TT 187 A | -2,58 |
| 26 | 9907 A | -2,58 |
| 27 | 7751 A | -2,58 |
| 28 | 917574 A | -2,68 |

| | | |
|---|---|---|
| 29 | 6545 A | -2,68 |
| 30 | 9726 A | -2,69 |
| 31 | TT 198 A | -2,71 |
| 32 | 8428 A | -2,74 |
| 33 | 8435 A | -2,80 |
| 34 | 8543 A | -2,82 |
| 35 | 9412 A | -2,84 |
| 36 | 8959 A | -2,86 |
| 37 | 2453 A | -2,92 |
| 38 | 9728 A | -3,01 |

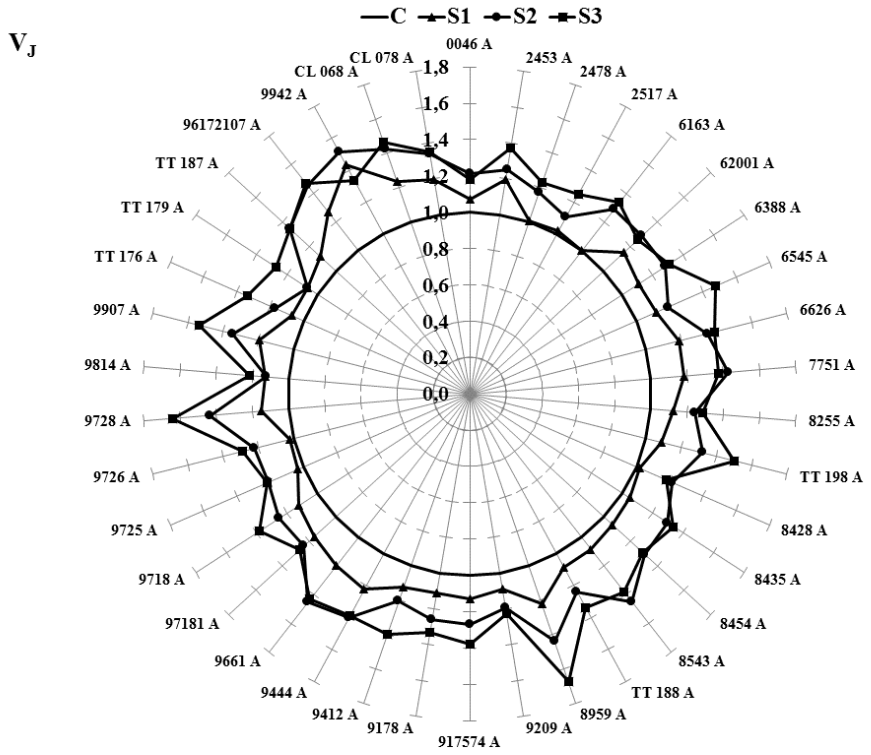


**Figure 1.** A radar-plot presentation of the changes in the relative variable fluorescence at the J-step ($V_J$) of sunflower inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

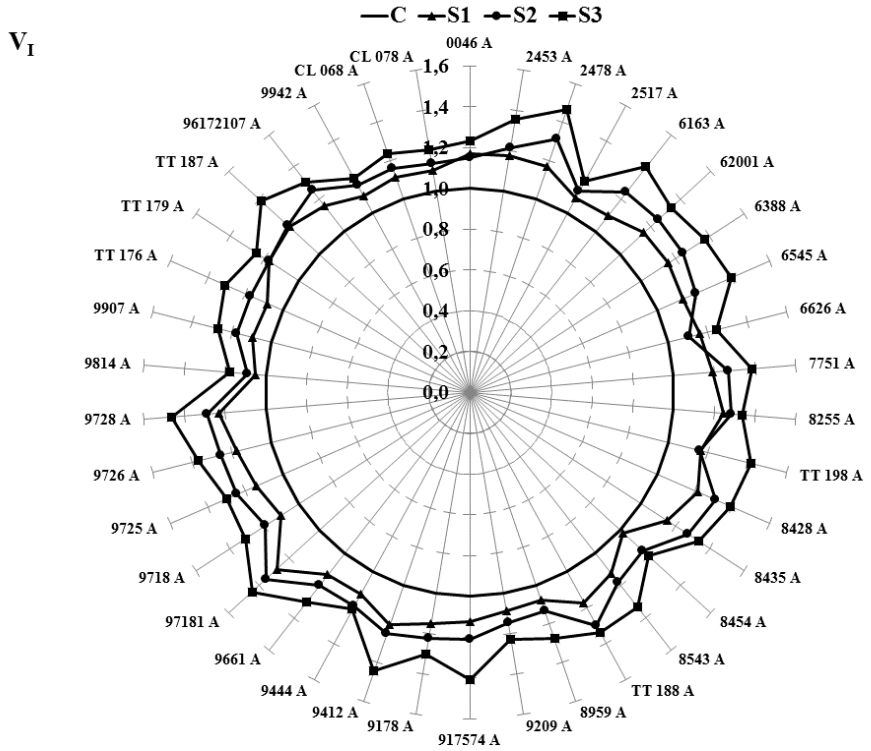


**Figure 2.** A radar-plot presentation of the changes in the relative variable fluorescence at the I-step ($V_I$) of sunflower inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

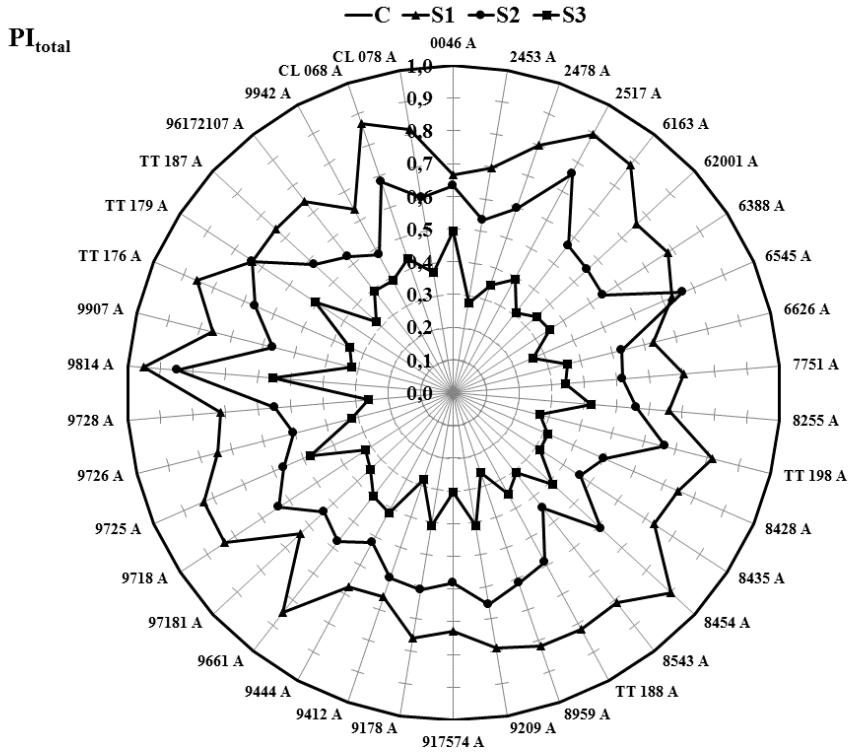


**Figure 3.** A radar-plot presentation of performance index ($PI_{total}$) parameter of dark-adapted sunflower leaves exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

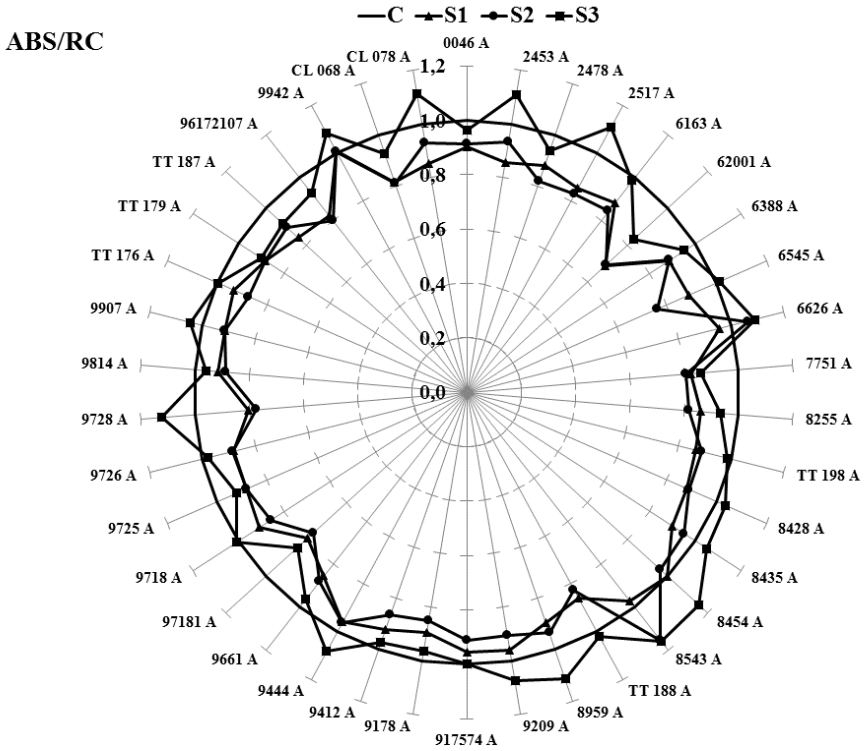


**Figure 4.** A radar-plot presentation of ABS/RC, effective antenna size of an active reaction centers, in the inbred lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

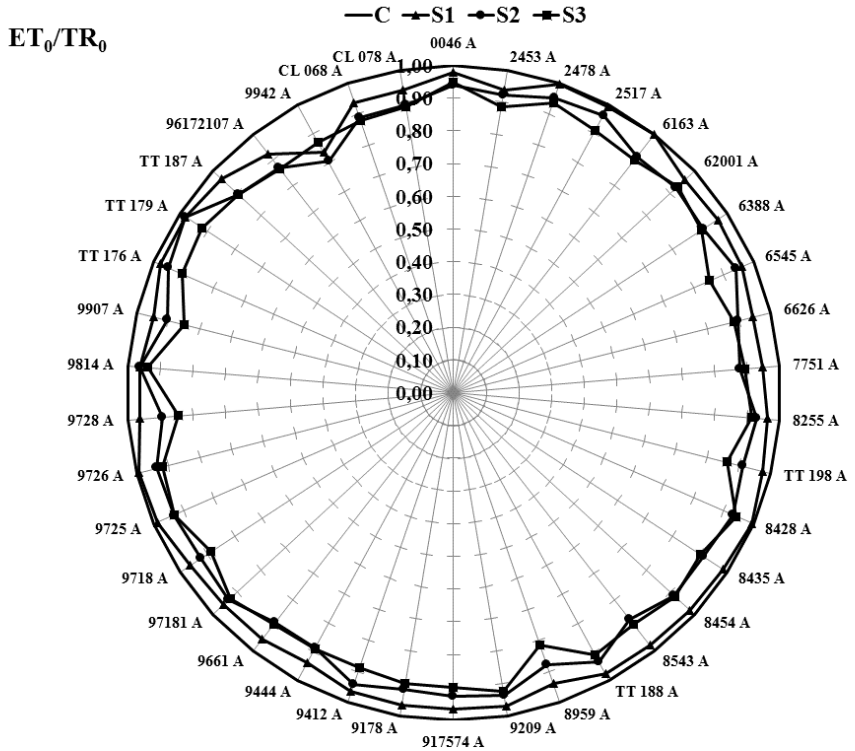


**Figure 5.** Drought stress effect on the efficiency with which the energy of a trapped exciton is converted into the electron transport beyond $Q_A$ ($\psi_o$=$ET_0/TR_0$) of inbred lines. All values of stress treatments are normalized by the values of their controls (control value: 1).

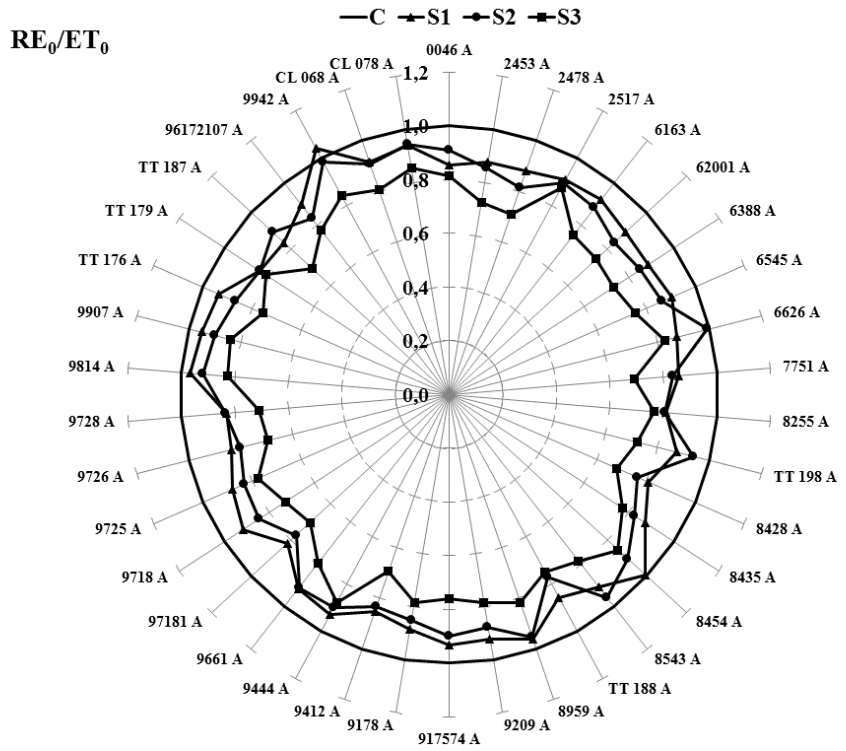


**Figure 6.** Drought stress effect on the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors ($\delta Ro = RE_0/ET_0$) of inbred lines. All values of stress treatments are normalized by the values of their controls (control value: 1).

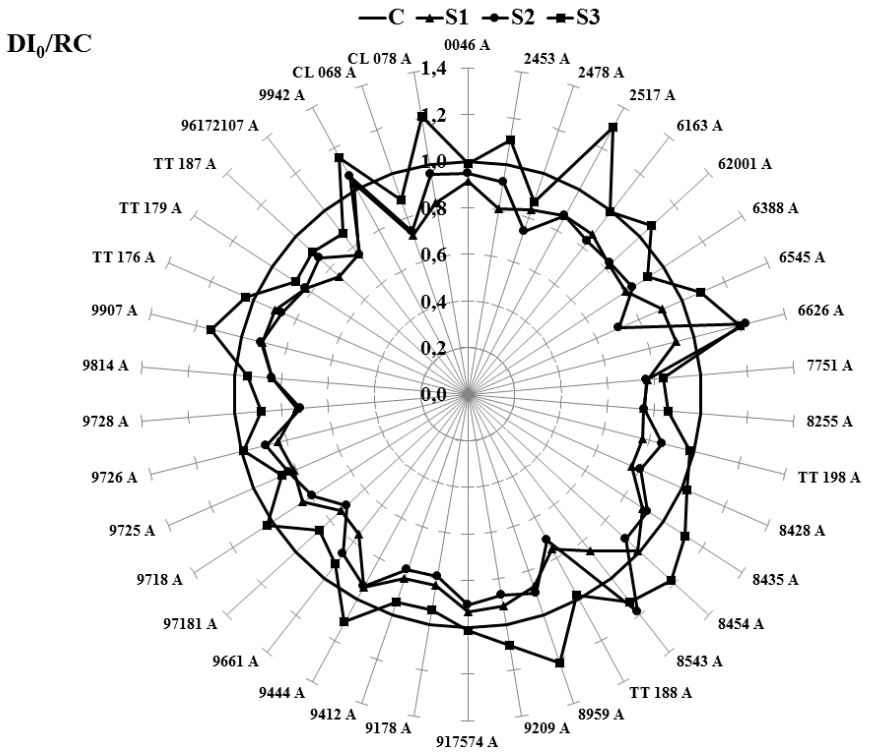


**Figure 7.** A radar-plot presentation of the flux of dissipated excitation energy per RC ($DI_0/RC$) of sunflower lines exposed to drought stress. All values of stress treatments are normalized by the values of their controls (control value: 1).

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# PHYSIOLOGICAL VARIABILITY OF SUNFLOWER DOWNY MILDEW CAUSAL AGENT, PLASMOPARA HALSTEDII, IN IRAN

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## ABSTRACT

Sunflower downy mildew caused by *Plasmopara halstedii*, is considered as one of the most important diseases in majority of the crop production areas. The use of resistant varieties and hybrids is an effective method to avoid its damage. Because of obligatory characteristics of the pathogen, it is exposed to ecological and physiological pressures ending to appearance of new physiological races. To obtain reliable and consistent resistances to the disease, monitoring of such variations are required. In this study we collected the isolates of the fungal agent and mass-produced them employing whole seedling immersion method. By using the same inoculation technique, sunflower downy mildew differential lines were inoculated and then evaluated for systemic infection as susceptible reaction. The results demonstrated physiological similarity of the isolates and also existence of race number 100 in Iran.

**Key Words :** Sunflower, downy mildew, physiological races.

# CHANGES IN THE PATHOGENIC COMPOSITION, ATTACKING THE OIL SUNFLOWER IN BULGARIA.

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## ABSTRACT

The sunflower development has been greatly hindered by the sunflower diseases. In the past ten years, we have been monitoring a shift from diseases that have been labeled highly significant, to such with a more sporadic nature. Due to the purposeful breeding work, the scientific community has created hybrids, resistant to leaf pathogens such as: gray spots (Phomopsis helianthi Munt.-Cvet. et al.), mildew (Plasmopara helianthi Novot.) and parasite broomrape (Orobanche cumana Wallr.). By contrast, the extreme temperature heights, during the vegetation period, well reduced the development and distribution of the black spots (Phoma macdonaldii Boerema). The climate change led to a high peak in the brown spots (Alternaria sp.) and charcoal rot (Macrophomina phaseolina (Tassi) Goid advancement. Every past year we notice an increase on the macrophomina attacks. Research shows that the infection in some selection materials can go as high as 50%. This tendency of pathogenic adjustment requires a rapid restructuring of the selection program to prevent declines in the production of sunflower.

**Key words**: sunflower, climate, pathogens.

## INTRODUCTION

The Earth's climate is in constant change, and so has the crops' development conditions. A crop moves from one phase to another in its development, as a result from reaching certain temperature sums. In the recent years we have been constantly speaking of a drastically changing climate, mainly referred to as the Global Warming. (Aleksandrov V. et all. 2010) has made an extensive research on the climate changes in the last few decades in Bulgaria. Some conclusions drown from this research are that the rise of air temperatures during the XX century has been the highest in comparison with previous centuries, as the 1906-2005 year period, the medium air temperature has been 0.74$^{o}$ C higher. The year with the highest temperatures is 2009. From the beginning of XX century, the rain over North Europe has risen with 10 to 40%, while the rain over some regions in South Europe (Bulgaria amongst them) has declined up to 20%. The most notable drought was during the year 2000. In some regions the agrometherological conditions has caused a decline in the vegetation period up to and below 90 days. Those regions include Dobrudzha and the south regions of northwest Bulgaria. The data from the phenological observations suggests that plants vegetation gets ahead of its normal course with 7-15days in the different climate regions, which without a doubt, proves that the climate has warmed during the last 30 years. The rise of temperature and diminution of rain has greatly affected the pathogenic composition attacking the crops, sunflower included. Growth cycle of these pathogens is closely associated with both the temperature and the atmospheric and soil moisture (Mari M and C.Martini, 2015), (M.Pautasso et al. 2012). The purpose of this study is to track changes in the pathogenic composition, attacking the oil sunflower in Bulgaria over the past two decades.

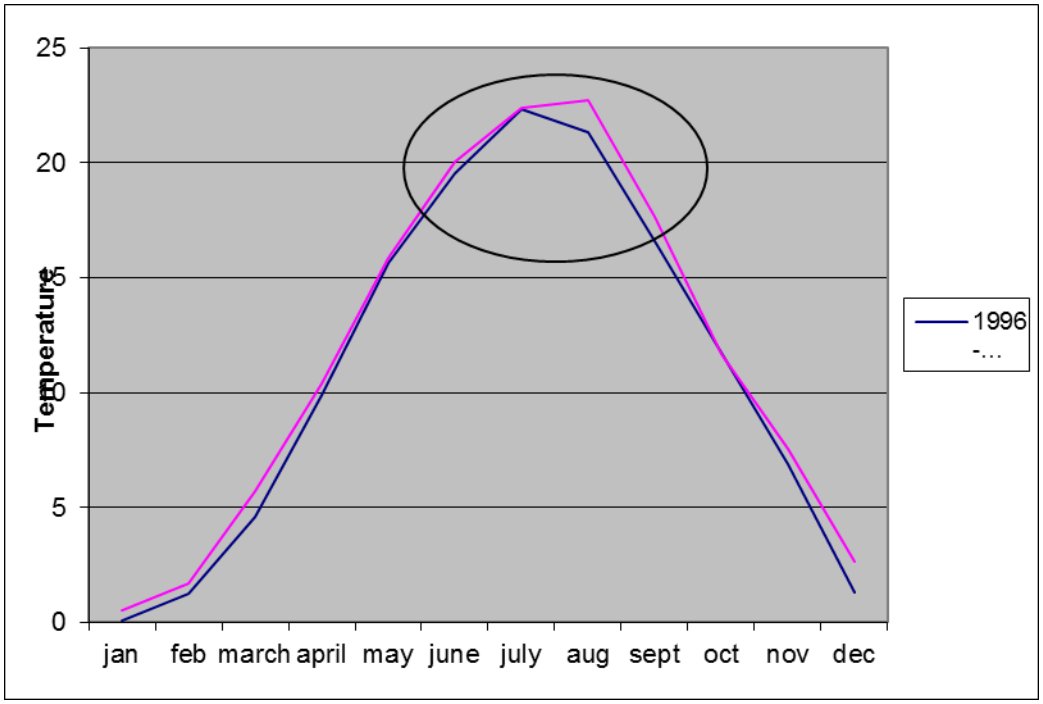
## MATERIAL AND METHOD

The investigation was carried out in artificial infection field of Doubrudja Agricultural Institute. During the vegetation period we have established the extent and the type of damage caused by economically important diseases. When a new set of diseases appear, they are registered in the appearing country, and scientific community begins their reporting starting the next year. The data used for the period 1996-2015, is taken from the annual reports of the author (unpublished data). Data for temperature and precipitation fallen is divided into two decades 1996 – 2005 and 2006 – 2015. They are obtained from the weather station located on the territory of Doubrudja Agricultural Institute - General Toshevo.

## RESULT AND DISCUSSION

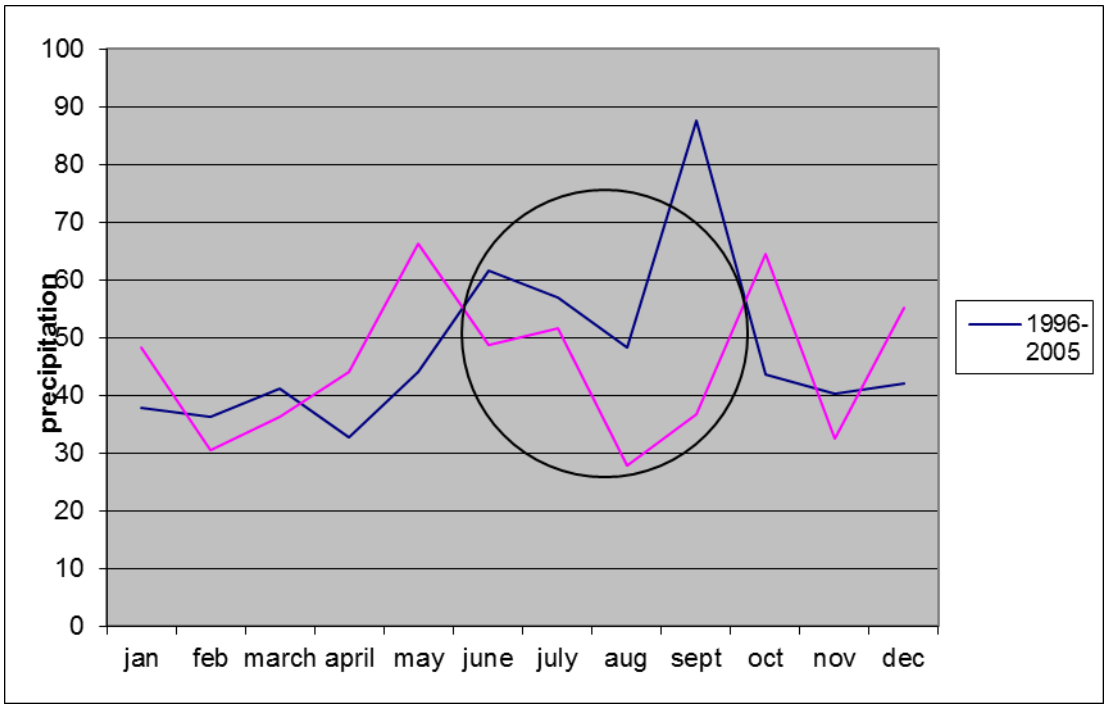
It is a fact that climate change has direct effects on the plant pathosystems. In the last two decades, the DZI science department has estimated a temperature increase (approximately 0.8°C-1°C) and soil moisture decrease, especially in the active vegetation period of sunflower when it is most prone to diseases attack. Plant pathologists have always considered environmental influences in their studies of plant diseases: the classic disease triangle emphasizes on the interactions between plant hosts, pathogens and the environment. (Garrett 2008; Klopfenstein et al. 2009; Grulke 2011 Coakley (1995) stated that disease development may increase, decrease or remain stable depending on the host-pathogen interaction. Any change in the ecosystem can affect plant diseases, as plant disease is the result of the interaction between a susceptible plant, and a virulent pathogen and the environment.

Fig.1 Average monthly temperature for the periods 1996-2005 and 2006-2015




When we examine two decades of data and compare the temperature and the precipitation during the sunflower's active vegetation (June, July, August), we clearly notice a tendency of temperature rise and reduction of precipitation in the 2006 – 2015 time period. This enviornmental change has let to shift in the sunflower's pathogenic composition.

Fig.2 Amount of precipitation in the periods 1996-2005 and 2006-2015




Moreover, some aspects associated with climate changes, such as the increase of temperature and changes in precipitation and moisture can have some effects on the fitness (number of generations, the sexual reproduction) of plant pathogens, extending the amount of time available for their reproduction and dissemination.

Table 1. Changes occurred in the distribution and aggressiveness of pathogens on sunflower

| Fungy | 1996 - 2005 | 2006 - 2015 |
|---|---|---|
| *Plasmopara helianthi* | strong attack in the field | Decreased attack on pathogen |
| *Phomopsis helianthi* | Medium to strongly attack | Reduce the intensity of attack |
| *Botritys cinerea* | Average intensity of the attack usually at the end of the growing season | Long and hot autumn with a single infested plants |
| *Alternaria sp.* | Medium attack | Moderate to severe infestations in some years |
| *Phoma macdonaldii* | Medium attack | Moderate to severe infestations in some years |
| *Albugo tragopogonis* | Singal plants | Spread throughout the country |
| *Puccinia helianthi* | From low to middle attack | Medium to strongly attack |
| *Rizopus sp* | Low attack | Increased severity of pathogen |
| *Macrophomima phaseolina* | Low attack | Increased severity of pathogen |
| *Verticilium dahliae* | Low attack | Increased severity of pathogen |

*Plasmopara helianthi* is an important disease on sunflower in Bulgaria. The last decade its primary appearance on sunflower fields has decreased because of the presence of effective fungicides, but the secondary infection, by the same pathogen, is commonly observed. Probably the climate change affects the host's biology and this indirectly influenced its response to pathogen attacks. Probably higher temperatures produce an elongation on the vegetative season and the consequent increase of secondary infections on leaves. The same result was observed by (Richerzhagen et al., 2011) in *Cercospora beticola* causing leaf spot on sugar beet in southern Germany. (Richerzhagen et al., 2011) suggest that due to an annual mean temperature increase by approximately 0.8°C-1°C in the last century the leaf spot attacks has risen.

According (Coakley et al., 1999) higher winter temperatures might increase pathogen survival on crop residues accumulating the amount of initial inoculum to infected subsequent crops. It is not excluded that this is due to severe attack by *Alternaria sp.* Our deductions and results are similar. We have observed amplification in the procent of attacks during the last two decades. (Encheva V, 2007).

The increase of temperature contributes to the spread of pathogens in some new geographical areas, where the pathogens can encounter new potential hosts. Initially we observed the disease *Albugo tragopogonis* Schr. ( *Encheva and all*.2000 ) in *2000.* During the last decade we estimated its attack on the whole Bulgarian territory. The same goes to another disease spread in Bulgaria: *Rhizopus sp*. (Encheva V and N.Nenov 2004).

The increased temperatures in winter and spring can assist the maturation of ascospores and their release, forcing an early start of the disease management. The general increase in temperature produces an extension of the vegetative season, exposing crops to higher infections. (*Phoma macdonaldi* and *Puccinia sp*.). This leads to a divergence cycle of both the disease and the host plant development. The climate change largely influence the manifestations of one or more fungal diseases.

In recent years, almost all sunflower vegetation goes by extremely high temperatures. They directly affect the development of pathogens in crops. Such widespread diseases, mainly occurring in areas with hot climate, are *Macrophomima phaseolina, Verticilium dahliae , Rizopus sp* . etc. Meanwhile the new climate situation limits the emergence of diseases such as *Phomopsis helianthi, Botritis cinerea.* There are fungal diseases like *Alternaria* that do not respond notable to weather conditions. Models predict in the mid-term a lower impact of oilseed rape diseases such as *Leptosphaeria maculans* and *Pyrenopeziza brassicae* (Fitt et al. 2011). The climate increase in Northern Germany, for example, facilitates the oil seed rape pathogens such as *Alternaria brassicae*, and *Sclerotinia sclerotiorum.* Indeed these new conditions not only threaten plant health but may in some cases exterminate the plant itself. It is predicted that *Verticillium longisporum* will be favoured by average increase in temperatures, particularly when taking into account a long-term (2071–2100) view (Siebold and von Tiedemann 2012).

## CONCLUSIONS

The impact of climate change on plant diseases requires more research. The change of climate could alter stages and rates of development of the pathogen, modify host resistance and thus lead to transitions in host-pathogen interaction. Climate changes mostly affect agricultural production. Research on the climate change impact on plant disease has led to a new aim: to create a drought-resistant sunflower hybrid with genes that control diseases, conducive to high temperatures and low soil moisture.

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# VARIATION IN AGGRESSIVENESS OF *PHOMA MACDONALDII* ISOLATES FROM THREE BALKAN COUNTRIES AND UKRAINE

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## ABSTRACT

*Phoma macdonaldii* is a ubiquitous pathogen, affecting sunflower by premature leaf senescence, stem cortical tissue necrosis and basal stem girdling. Sunflower genotypes expressing partial resistance have been reported. Currently, disease has reemerged as a threat to sunflower production in some sunflower cropping areas. To estimate variability in pathogen population, sunflower stems with disease symptoms from Serbia, Turkey, Romania and Ukraine were sampled. Total of 54 isolates was used for inoculation of four inbred lines differing in resistance to phoma black stem. Results from scoring of sunflower inbred lines seven days after inoculation showed significant differences in disease severity measured by percentage of cotyledon petiole necrosis. Significant difference was detected both among isolates and genotypes. Isolates were segregated in five clusters. Five isolates were found to be highly aggressive based on disease severity. The least aggressive were seven isolates, producing mild symptoms on all tested genotypes. Majority of tested isolates lead to complete necrosis of inoculated plant part of the most susceptible genotype and mild symptoms of other three genotypes. Isolate aggressiveness was not correlated with geographic origin. In conclusion, significant variability among pathogen isolates was confirmed with several isolates distinguished as highly aggressive. This research could assist in breeding process for resistance to phoma black stem.

**Keywords:** sunflower, *Phoma macdonaldii,* aggressiveness

## INTRODUCTION

Diseases are a major constraint in sunflower production. Phoma black stem, caused by pathogenic fungus *Phoma macdonaldii*, is widely distributed disease, usually considered to have limited impact on sunflower yield and quality (Gulya et al., 1997). The most distinguishing symptoms of disease appear in form of black lesions on stem, elliptical in shape and commonly 5-10 cm in length (Marić and Schneider, 1979). Symptoms can develop at stem base and in time girdling of stem may result in premature ripening (Donald *et al*., 1987).

Severity of disease depends on sunflower genotype and up to date no complete resistance to phoma black stem has been found. Sunflower genotypes significantly differ in susceptibility (Roustee et al., 2000a; Bert et al., 2004). Tolerant genotypes were found in cultivated and wild sunflowers (Darvishzadeh et al., 2010; Larfeil et al., 2010). Disease development differs spatially and temporally as a result of influence of environmental factors and cultural practices (Sessau et al., 2010; El Sayed and Marić, 1981). In addition, variability among sunflower genotypes is complemented with differences in isolate aggressiveness

(Roustaee et al., 2000b). Most recently, virulence variability of pathogen was reported in Argentina (Lazzaro *et al.*, 2012).

The objective of this study was to determine aggressiveness of *P. macdonaldii* isolates collected in Serbia and compared these results with aggressiveness of isolates from three countries where sunflower is extensively cultivated.

## MATERIAL AND METHOD

*P. macdonaldii* isolates were collected in 2012, across three regions in Serbia, and received from Ukraine, Romania and Turkey. Four sunflower inbred lines (CMS-1-122, ROD-DI-111, VL-A-8, DOP-32-08), differing in resistance to phoma black stem were selected based on previous research (Dedić *et al*., 2012). Sunflower seed was surface sterilized in 1% solution of NaOCl and sown in plastic containers 9x9x9 cm in size, and filled with peat. Four plants grown in each container served as replication. Experiment was set in three replications. Temperature during experiment was maintained at 22/18 °C during 16/8 h photoperiod. Inoculation of plants, with fully developed first pair of leaves, was done following method described by Roustaee *et al.* (2000b). Cotyledon petioles were inoculated with 20 µl of *P. macdonaldii* picnospore suspension, concentration $10^6$ picnospores/ml. Seven days after inoculation disease severity was assessed using scale 1-9 (Roustaee *et al*., 2000b). For each line and isolate median value was calculated and isolates were clustered using software PAST (Hammer *et al.*, 2001).

## RESULTS AND DISCUSSION

Results after inoculation of four inbred lines shows significant difference in susceptibility to disease with median values ranged from 1 to 9 (Figure 1). Significant variation of disease severity was observed among isolates expressed in large interquartile range particularly for inbred lines ROD-DI-111 and VL-A-8.

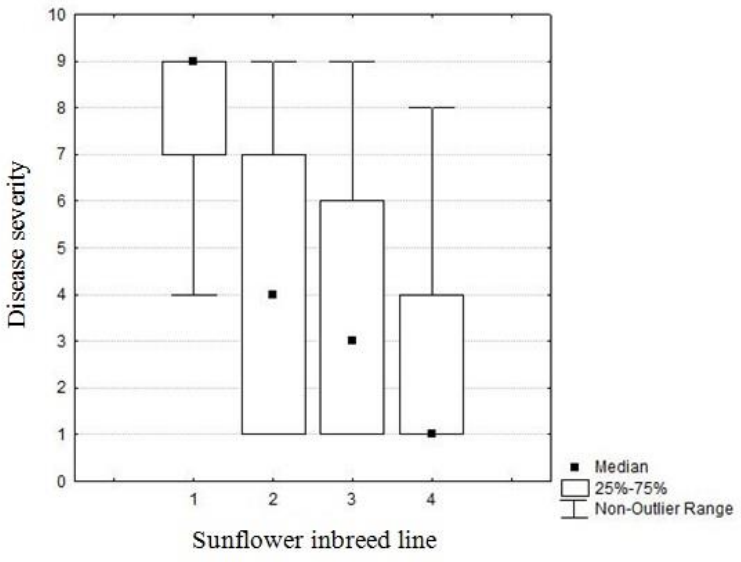


Figure 1. Median values and variation of Phoma black stem severity for all tested isolates on inbred lines CMS-1-122 (1), ROD-DI-111 (2), VL-A-8 (3) and DOP-32-08 (4)

Isolates were clustered in four distinct groups based on disease severity of inoculated inbred lines (Figure 2). The first group was consisted of five isolates which produced the most severe symptoms and consequently were considered to be highly aggressive. Out of this five isolates origins of two is same region in Serbia (SRB-R1S52, SRBR1S21), another two were sampled in Ukraine and one in Romania. The second group has two isolates which were characterized by high disease severity on inbred lines CMS-1-122 and DOP-32-08 and mild symptoms on other two inbred lines. The third group consisted of five low aggressive isolates. Isolates from the fourth and the largest group expressed high disease severity on genotype CMS-1-122, moderate disease severity on genotypes ROD-DI-111 and VL-A-8, and low disease severity on genotype DOP-32-08.

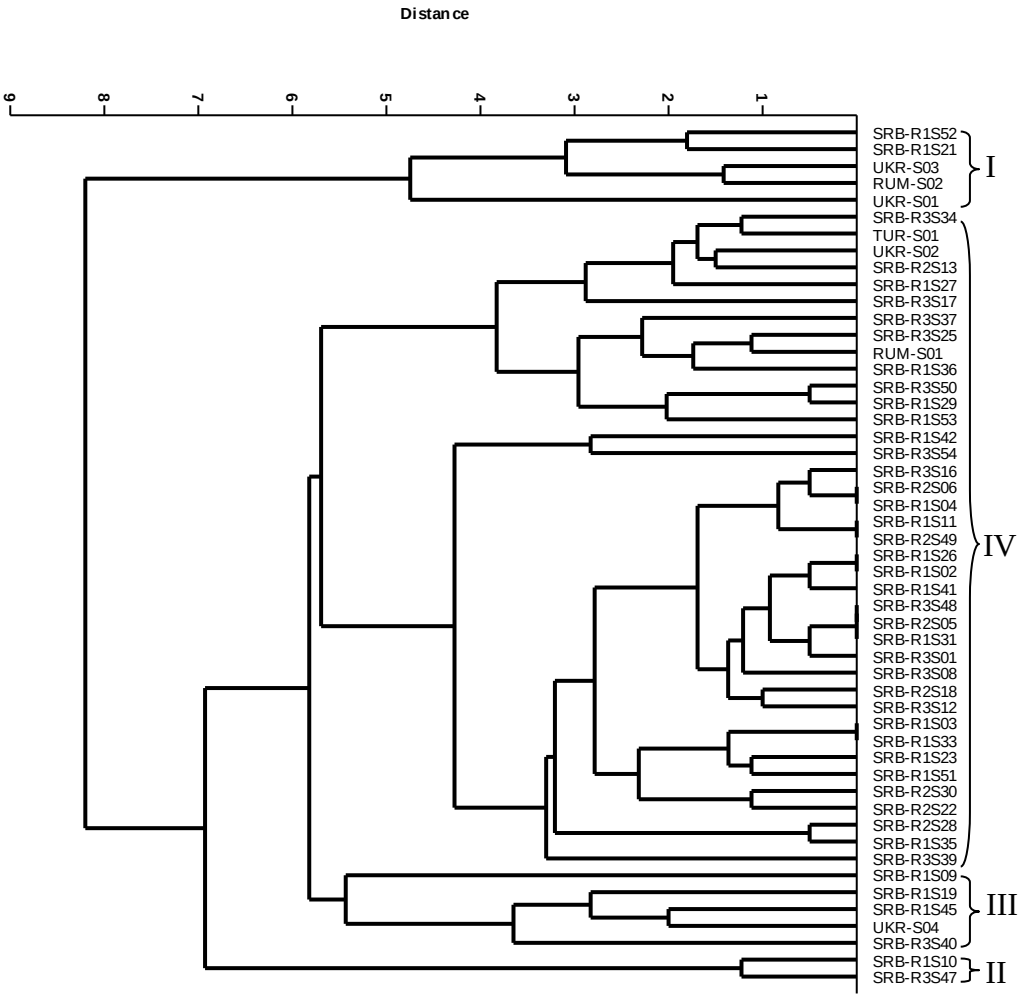


Figure 2. Dendrogram showing similarity and clustering of *P. macdonaldii* isolates based on their aggressiveness on four sunflower inbred lines

Considerable variability in aggressiveness was confirmed as a result of this research. Majority of tested isolates have similar pattern in disease severity on selected genotypes. However, a group of both high and low aggressive isolates was distinguished along with two isolates able of producing severe symptoms on the most resistant genotype. Isolates not originated from Serbia were not clustered based on aggressiveness. Most of these isolates proved to be highly aggressive. However, number of isolates from other countries was small

and this conclusion needs to be verified on larger sample of pathogen population from that areas. Difference in aggressiveness among *P. macdonaldii* proved in this research was also confirmed by other researchers. Most data comes from France where this disease is considered highly damaging (Mirleau-Thebaud *et al.,* 2011). Larfeil *et al*. (2002) determined five pathotypes based on stark differences in disease severity, following inoculation of ten sunflower inbred lines. Rostaee *et al*. (2000a) presented considerable variability among isolates in various traits including aggressiveness. Similar differences were found after testing isolates of pathogen in Argentina (Lazzaro *et al.,* 2012). In addition, highly significant genotype-isolate interaction in *P. macdonaldii* – sunflower pathosystem was reported (Darvishzadeh *et al.,* 2007; Maleki and Darvishzadeh, 2014).

In conclusion, patterns of reaction of four inbred lines to disease revealed differences in aggressiveness, with 5 out of 54 tested isolates regarded as highly aggressive. Isolates with similar aggressiveness did not cluster according to geographic origin. Determination of pathogen variability will provide selection of *P. macdonaldii* isolates suitable for breeding programs.

## ACKNOWLEDGEMENTS

This work was supported by Ministry of Education and Science, Republic of Serbia, project TR 31025.

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# SUNFLOWER DISEASES IN NORTHERN GREECE

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## ABSTRACT

Sunflower has been cultivated extensively in Greece since 2010, mainly for the production of biofuels. The major region of cultivation is Northern Greece, covering approximately 60.000 hectares. Twelve diseases and three non-parasitic disorders were detected by the author during the 2011-2015 period. Based on incidence rates and severity, the diseases are divided into two categories: a) major diseases, which include Downy mildew, Septoria leaf spot, Alternaria leaf blight, Phoma black stem, Charcoal rot and Phomopsis stem canker and b) minor diseases, which include Sclerotinia wilt, Rust, Powdery mildew, Rhizopus head rot, Bacterial leaf spot and Bacterial stalk rot. Seed scorch, Leaf scorch and Bract necrosis were the non-parasitic disorders observed. The five-year survey showed that Phoma black stem and Charcoal rot were the most significant and prevalent (77% and 75% incidence rates respectively in the 2011-2015 period) diseases of sunflower in Northern Greece and they are considered– especially Charcoal rot – to be the cause of premature ripening syndrome. The leaf disease exhibiting the greatest incidence rate (31% during the 2011-2015 period) was Septoria leaf spot.

**Key Words :** sunflower

# HELIAPHEN : A HIGH-THROUGHPUT PHENOTYPING PLATFORM TO CHARACTERIZE PLANT RESPONSES TO WATER STRESS FROM SEEDLING STAGE TO SEED SET

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## ABSTRACT

Characterization of plant morphological and physiological responses is a limiting step to breed crops adapted to drought-limiting conditions. Automation of plant management on a phenotyping platform overcomes it by allowing large scale experimentation with yet accurate and individual plant monitoring. In response to both genetic and eco-physiological experimentation requirements, we developed the HELIAPHEN platform. This unique outdoor platform can host 1300 plants, such as sunflower, in 15L pots. It allows plant growth in climatic conditions similar to field, as well as a precise and automated monitoring of plant water consumption thanks to a prototype robot. Its primary functions are to move autonomously on the 600m² platform, and to treat each pot at its location (including weighing and watering up to a targeted weight). Beyond these functions, the robot takes at each handling, plant images from multiple angles with four cameras, to follow the evolution of morphological traits along with the description of the water status. In addition, a ultrasound radar measures automatically plant height and a laser measures stem diameter at the plant basis. These secondary functions are currently improved with new captors such as a light curtain and a 3D laser in order to reconstitute a 3D representation of the plant. To validate the meaning of the HELIAPHEN outputs, we confirmed the impact of drought stress managed with the robot on seed weight, number and thousand kernel weight (TKW). Furthermore, we observed a correlation between field and HELIAPHEN data for TKW and seed number observed on 45 sunflower hybrids.

**Key Words :** robot, drought, transpiration, growth, imaging

# INDUCED RESISTANCE IN SUNFLOWER AGAINST WHITE ROT (*SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY) AND DOWNY MILDEW (*PLASMOPARA HALSTEDII* (FARL.) BERL. ET DE TONI)

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## ABSTRACT

The main diseases of sunflower, such as white rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) and downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni) cause severe yield losses worldwide. Controlling these pathogens by available tools is rather difficult because of the poliphag character (*S. sclerotiorum*) and the high genetic variability *(P. halstedii*) of the parasites. Recently, a major interest has focused on the relevance of alternative protection methods, such as induced resistance against pathogens. In our work we tested the effects of some chemical inducers (benzothiadiazole /BTH/, isonicotinic acid /INA/, beta-aminobutyric acid /BABA/) as well as biological activators (arbuscular mycorrhizal fungi /AMF/ and Trifender /*Trichoderma asperellum* T1/) against white rot and downy mildew in four sunflower genotypes (cv. Iregi szürke csíkos, P63LE13, PR64H41 and Croplan DMR) in glasshouse. BTH was also investigated in a field experiment. Applied alone and in combination, Trifender, AMF and most efficiently BTH decreased downy mildew symptoms (sporulation) in cv. Iregi. Inducers significantly reduced white rot development in cv. Iregi (AMF fungi) and in Croplan (BABA, INA, BTH). In the field experiment with BTH the development of sclerotinia rot was restricted at the beginning in cv. Iregi and hybrid Croplan but not in hybrid PR64. Ratio of dead plants, however, was significantly lower in all sunflower genotypes treated with BTH and infected with the fungus compared to control plants. According to our results, application of these activators may be considered in future plant protection against sunflower diseases.

**Key Words :** SAR, *Sclerotinia* rot, sunflower downy mildew.

# A REEVALUATION OF MYCELIOGENIC GERMINATION OF SCLEROTIA FOR *SCLEROTINIA SCLEROTIORUM* STRAIN SUN-87

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## ABSTRACT

Basal stalk rot of sunflower is an economically important and rather unique disease among crops that are susceptible to *Sclerotinia sclerotiorum*. This disease is the result of myceliogenic germination of sclerotia whereby the vegetative hyphae infect the sunflower below the soil level. In contrast, sunflower head rot and similar diseases of susceptible crops result from carpogenic germination to produce airborne ascospores that infect above ground senescent or wounded tissues. We initiated research on several factors reported to affect sclerotia germination as a prelude to comparing transcriptomes associated with myceliogenic and carpogenic germination. Specifically, we reevaluated the effects of inoculum development temperature, sclerotia development temperature, conditioning temperature, conditioning of hydrated and desiccated sclerotia, and the duration of sclerotia desiccation on germination of Sun-87 sclerotia, largely as outlined by Huang (1991), Huang and Kozub (1993), and Huang et al. (1998). We were not able to use conditioning temperature to clearly differentiate myceliogenic and carpogenic germination (-20 vs. $\geq 0.5^0$C), as reported by Huang (1991), using either hydrated or desiccated Sun-87 sclerotia. Additionally, we were not able to verify that a low inoculum production temperature was the main factor affecting carpogenic germination of Sun-87. Rather, a low temperature during inoculum and/or sclerotia production enhanced germination. Finally, we were not able to verify that myceliogenic germination of Sun-87 occurred most readily when sclerotia formed at 20-25$^0$C were desiccated prior to germination. Desiccation almost always resulted in carpogenic germination, albeit at a low level relative to germination of hydrated sclerotia. Additional experiments are in progress to discover a reliable and non-confounded method that clearly differentiates myceliogenic and carpogenic germination.

**Key Words :** Sclerotinia sclerotiorum; white mold; stalk rot; head rot; myceliogenic germination; carpogenic germination; disease; pathology

# SEED PRIMING APPLICATION EFFECT ON ALLEVIATION OF DROUGHT STRESS IMPACTS DURING GERMINATION IN SUNFLOWER HYBRIDS (*HELIANTHUS ANNUUS* L.)

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## ABSTRACT

In order to study the effect of different seed priming techniques on germination and early growth of sunflower under drought stress conditions, a factorial experiment based on completely randomized design with 3 replications was conducted in the laboratory of Seed and Plant Certification and Registration Institute. Seeds of two sunflower cultivars; Azargol and Hysun-36 were pre-treated with 5 treatments including: 2 osmopriming concentrations of Potassium Nitrate (KNO3); 500 and 1000ppm, two hydro-priming with distilled water in two durations of 12 and 18 h and a control treatment without priming. After priming, seeds were placed on different osmotic drought conditions for germination test and early growth evaluation. Osmotic conditions were provided by PEG-6000 in 3 osmotic potential levels; -0.3, -0.6 and -0.9 MPa and one control condition of 0 MPa. Results showed that, The lowest seed germination percentage and early growth occurred at -0.9 MPa for both cultivars and priming with 1000 ppm KNO3 increased seed adaptation to osmotic conditions because the highest germination and growth under osmotic condition observed in this treatment. Hysun-36 showed to be more drought tolerant so that highest germination and growth in osmotic dry condition demonstrated for this cultivar. There were no significant difference in seed germination and early growth performance under osmotic drought between hydro-priming 18h and non-primed control. This results revealed that to gain a better germination and seedling stablishment in dry cultivation, osmo-priming with 1000 ppm KNO3 may be beneficial.

**Keywords**: sunflower, seed priming, germination percentage, drought stress and seedling vigor index

## INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil seed crops in Iran. It is a high yielding oilseed crop, but under scarce conditions, the yield is very lower than its real potential. Among the factors responsible for the low yield, imbalance use of fertilizer, improper plant protection, poor growth and sub optimum plant population are rather important. Suboptimum plant population generally results from poor and erratic germination (Barsa et al., 2003). Recently, salt and drought stress are perhaps the two most important abiotic stresses that limit plant growth and development(Elhafid et al., 1998). A good strategy is the selection of cultivars and species for salinity and drought conditions(Ashraf et al., 1992). But an alternative strategy for the possibilities to overcome salt and drought stresses is by seed treatments with hydro priming or other treatments(Yagmur and Kaydan, 2008). Seed priming is now a widely used commercial process that accelerates the germination rate and improves seedling uniformity in many crops(Halmer, 2003; Taylor and Harman, 1990). Priming allows some of the metabolic processes necessary for germination to occur without germination take place. The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, rice, soybean and sunflower (Parera and Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini, 2003; Yari and Sheidaie, 2011; Sadeghian

and Yavari, 2004). Seed priming with Potassium Nitrate ($KNO_3$) had shown good potential to enhance germination, emergence and seedling dry weight of Sunflower(Kaya et al., 2006; Singh and Rao, 1993**)**, corn(Basra et al., 1989**)** and soybean(Saadateyan et al., 2009). Hydropriming method has also been used successfully in sunflower(Kaya et al., 2006; Saadateyan et al., 2009), wheat(Harris et al., 2001), Rice(Yari and Sheidaie, 2011) and cotton(Casenave and Toselli, 2007). Moreover hydro priming increased germination and seedling growth under salt and drought stresses(Kaya et al., 2006; Saadateyan et al., 2009).

The present study was, therefore, carried out with the objective to evaluate the effects of seed priming treatments under drought stress conditions on germination and the seedling growth of hybrids sunflower.

## MATERIALS AND METHODS

The experiment was conducted in the laboratory of the department of seed and plant certification &registration Institute, Iran in 2011. Experimental units were arranged factorials in a completely randomized design (CRD) with three replications.

**Seed material:** Seed materials of Sunflower hybrids(Hysun-36 and Azargol), which selected on the basis of their area that planted in Iran.

**Priming techniques:** Priming media were used such as distilled water, 1000 ppm Potassium Nitrate (KNO3); 500ppm Potassium Nitrate ($KNO_3$); and control. All priming media were prepared in distilled water. Seed was fully immersed in $KNO_3$ priming media at a temperature of 25°C for durations of 2 hours and immersed in distilled water of 25°C for durations of 12 and 24 hours under dark conditions. Seed were removed from priming media at the same time, then rinsed thoroughly with distilled water and lightly dried using blotting paper and then allowed to dry on paper towels at room temperature. Control treatment consists of untreated seed.

**Osmotic stress of $PEG_{6000}$:** Three drought stresses with different osmotic potentials of -0.9,-0.6 and -0.3MPa were arranged as described by Michel and Kaufmann(1973). The osmotic potential of control solution was 0 MPa. The osmotic potentials of $PEG_{6000}$ were read with a Wescor Vapour Pressure Osmometer-5520.

**Seed germination test and seedling growth:** The treated and untreated seeds were then transferred to Petri dishes (50 seeds per Petri dish with three replications) containing two (whatman No.2) filter paper moistened with 10ml of control solution or the same solution added with PEG6000**.** Petri dishes were placed in germinator at 25±1°C under dark conditions. The Petri dishes were controlled in one day intervals for solutions content. Germination was recorded daily up to day 7 after the start of the experiment. A Seed was considered germinated when radical emerged by about 2mm in length (ISTA, 2003).

Mean germination time, germination percentage, germination rate and vigor seedling index were calculated as described in Ellis and Roberts(1981).

$$MGT\ (day) = \sum NiDi/N \qquad (1)$$

Where *Di* is the number of days after sowing, *Ni* is the number of seeds germination on $i^{th}$ day, N is the total number of germinated seeds.

Germination Percentage (GP) was calculated as $GP = \frac{\text{Total seeds germination}}{\text{Total number of seeds}} \times 100$ (2)

$$\text{Germination Rate (GR)} = \sum Ni/\sum TiNi \qquad (3)$$

Where Ni is the number of newly germinated seeds at time Ti.

The Seedling Vigor Index(SVI) was calculated as the product of seedling dry weight by germination percentage.

Seedling Vigor index(VI)=SDW×GP (4) Where SDW is seedling dry weight at the end of test and GP% is the final germination percentage. Final germination percentage, seedling length, radical length, stems length, seedling dry weights were recorded 7 days after cessation of the experiment. MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983**)**. The significance between the means were compared using Least Significant Difference(LSD) values($P<0.05$).

**RESULTS**

**Germination:** Germination percentage was influenced by seed priming treatments and drought osmotic stress conditions. The results indicated that Hysun-36 hybrid showed a significantly less decline in germination percentage under drought stress conditions in comparison to Azargol. Germination percentage under drought stress (-0.3, -0.6 and -0.9MPa of PEG) was increased by Seed primed with $KNO_3$ and water(12h) compared to untreated seeds(Table1). However, hydro priming seeds for 18h indicated no significant differences with untreated ones in percent germination(Table1).

**Mean Germination Time:** A significant three-way Interaction (hybrid, seed treatment and stress) was found for MGT ($P<0.01$)(Table2). There was no significant difference in MGT among primed seeds under control drought stress condition (0 MPa PEG) for both Azargol and Hysun-36hybrids. Seed primed with 1000ppm $KNO_3$ reduced the time to start germination and MGT under -0.6 and -0.9 MPa osmotic potential of PEG compared with other treatments in Azargol hybrid. Also seed treated with 500 ppm $KNO_3$ and 1000ppm $KNO_3$ shortened the time to seed germination compared with other treatments in Hysun-36 hybrid. Whereas, hydro primed seeds for 18h had negative significant effect on MGT at (-0.3, -0.6 and -0.9 MPa) and -0.9 MPa of PEG in Azargol and Hysun-36hybrids, respectively (Table2).

**Germination Rate (GR):** A significant three-way Interaction(hybrid, seed treatment and stress) was found for GR ($P<0.05$). Seeds primed with 1000ppm $KNO_3$ and hydro priming enhanced rate of germination under control stress condition (0 MPa of PEG) in Azargol hybrid. Also seed treated with $KNO_3$(especially 1000ppm $KNO_3$) improved rate of germination at higher concentration of PEG for both Azargol and Hysun-36hybrids.

**Root length**: Results of comparison means of hybrid and osmotic stresses showed that Azargol hybrid had longer root length than Hysun-36 hybrid under control drought stress condition(0 MPa of PEG). In contrast; increasing concentration of PEG improved length of root in Hysun-36 hybrid. The result of this experiment showed that priming with 1000ppm $KNO_3$ and hydro priming(12h) increased root length under drought osmotic stress conditions when concentration of PEG increased.

**Shoot length**: Shoot length was significantly influenced by hybrid and osmotic stresses ($P<0.01$). Results of comparison means of hybrid and drought osmotic stress conditions showed that the highest shoot length was attained from Azargol hybrid under control drought stress condition(0MPa of PEG), but Hysun-36hybrid had longer Shoot length than Azargol hybrid under drought osmotic stress at -0.3 MPa of PEG. The results of comparison means of seed priming treatments and osmotic stresses indicated that in the conditions of 500 ppm $KNO_3$ and 1000ppm $KNO_3$ treatments under control drought stress condition(0 MPa of

PEG) the longest status was shown, but by increasing concentration of PEG, there were no significant differences in Shoot length among seed priming treatments(Data not shown).

**Seedling dry weight:** Results of comparison means of hybrid and drought osmotic stress conditions showed that Hysun-36hybrid had more Seedling dry weight in comparison to Azargol hybrid under drought osmotic stress conditions ($P<0.01$). Seedling dry weight was significantly affected by seed priming treatments. Seed subjected with 500ppm $KNO_3$, 1000ppm $KNO_3$ and hydropriming(12h) increased seedling dry weight compared with untreated seeds while hydro priming for 18h had negative effect on Seedling dry weight compared to untreated seeds(Table3).

**Seedling Vigor Index:** The results of comparison means of hybrid and osmotic stresses indicated that seedling vigor index of Hysun-36hybrid at -0.3 and -0.6MPa concentration of PEG was higher compared to Azargol hybrid. The results showed that by increasing drought osmotic stress, Hysun-36 hybrid have more potential resistance in germination stage compared with Azargol hybrid, under drought osmotic stress conditions. Results of priming media comparison means showed that higher seedling vigor index was recorded from applying 1000ppm $KNO_3$ priming treatment. The lowest Seedling Vigor Index was observed in untreated seeds. These findings indicated that primed sunflower seeds with $KNO_3$ could positively affect on seedling Vigor Index(Table 3).

## DISCUSSION

In many crops pre-soaking or priming causes improvement in germination and seedling establishment (Harris et al. 2001). Increases in the seedling correlated with higher water uptake by primed seed resulted in higher seedling growth. The beneficial effects of $KNO_3$ on germination were found in this study .Final germination was higher from1000ppm $KNO_3$, Suggesting no toxicity of $KNO_3$ due to ion accumulation in the embryo, which is in support with the earlier findings (Demir and Venter, 1999; Kaya et al., 2006; Singh and Rao, 1993). Also seeds primed with 1000ppm $KNO_3$ and hydropriming(12h) improved rate of germination under drought osmotic stress conditions. these finding are line with Mvale et al(2003) reported that osmopriming seed improved germination rate in sunflower seeds. Also osmopriming has been shown to activate processes related to germination, through affecting the oxidative metabolism such as increasing superoxide dismutase(SOD) and peroxidase (POD)(Jie, 2002). Moreover, the present study revealed that seed treated with 500ppm $KNO_3$ and 1000ppm $KNO_3$ shortened the time to seed germination compared with other treatments. These finding are in line with Demir and avaenter(1999) who states that seed primed with $KNO_3$ reduced MGT and had positive effect on germination percentage in sunflower seeds. In contrast, hydro priming seeds for 18 hour had negative effect on MGT under -0.9 MPa osmotic potential of PEG. This could be explained by more rapid water uptake than the amount of water for germination in these hybrids. Also the results showed that by increasing drought osmotic stress, Hysun-36hybrid have more potential resistance in germination stage compared with Azargol hybrid. Increasing concentration of PEG improved length of root in Hysun-36hybrid.These findings support the earlier work of Beckman et al.(1993), who reported that increasing in length of root in switch grass by seed priming treatments.

Table1. Effect of seed priming treatments on germination percentage of hybrids sunflower under drought stress conditions

| Seed priming treatments \ Osmotic potentials(MPa) | 0 | -0.3 | -0.6 | - 0.9 |
|---|---|---|---|---|
| Control | 96.17 a | 72.00 d | 41.67 g | 18.67 j |
| 500ppm ($KNO_3$) | 92.33 ab | 73.67 cd | 54.33 e | 34.33 h |
| 1000ppm ($KNO_3$) | 96.17 a | 78.83 c | 54.33 e | 46.17 fg |
| distilled water(12h ) | 94.67 a | 78.50 c | 49.67 ef | 27.67 i |
| distilled water(18h ) | 91.83 ab | 73.33 cd | 45.5 fg | 19.50 j |

*Means with same letter are not significantly different at *LSD (P<0.05*)

Table2. Effect of seed priming treatments on Mean Germination Time of hybrids sunflower under drought stress conditions

| | | Osmotic potentials(MPa) | | | |
|---|---|---|---|---|---|
| Hybrid | treatments | 0 | -0.3 | -0.6 | -0.9 |
| Azargol | Control | 1.69 P | 2.25 l | 3.24 h | 4.63 d |
| Azargol | 500ppm ($KNO_3$) | 1.64 P | 2.19 lmn | 3.22 h | 4.70 cd |
| Azargol | 1000ppm ($KNO_3$) | 1.67 P | 2.13 lmno | 2.95 i | 3.93 ef |
| Azargol | distilled water(12h ) | 1.63 P | 2.21 lm | 3.21 h | 4.68 cd |
| Azargo | distilled water(18h ) | 1.68 P | 2.61jk | 3.75 fg | 5.11 b |
| Hysun-36 | Control | 1.98 o | 2.75 ij | 4.0 1e | 5.31 b |
| Hysun-36 | 500ppm ($KNO_3$) | 1.98 o | 2.53 k | 3.76 fg | 4.84 c |
| Hysun-36 | 1000ppm ($KNO_3$) | 1.99 mno | 2.53 k | 3.64 g | 4.71 cd |
| Hysun-36 | distilled water(12h ) | 1.98 no | 2.92 i | 4.04 e | 5.3 b |
| Hysun-36 | distilled water(18h ) | 1.01mno | 2.95 i | 4.01 e | 5.65 a |

*Means with same letter are not significantly different at *LSD (P<0.05*)

Table3. Effect of seed priming treatments on seedling dry Weight (g) and Vigor index of sunflower

| Seed priming treatments | Seedling dry Weight(g) | Vigor index |
|---|---|---|
| Control | 25.79 b | 19.45 c |
| 500ppm ($KNO_3$) | 28.15 a | 22.5 ab |
| 1000ppm ($KNO_3$) | 28.44 a | 23.84 a |
| distilled water(12h ) | 27.64 a | 22.38 ab |
| distilled water(18h ) | 26.93 ab | 21.22 b |

* Means with same letter are not significantly different at LSD ($P<0.05$)

## CONCLUSIONS

Overall it could be concluded that suitable priming the sunflower seeds was 1000ppm $KNO_3$ resulted in higher germination percentage and seed vigor under drought osmotic stress conditions. Therefore, priming with $KNO_3$ may be an efficient method to overcome seed germination problems and to improve seedling growth in field, especially under drought conditions. Hydro priming for 18 h had negative effect on Seedling dry weight compared to untreated seeds. It was concluded that increasing of hydro priming time may have negative impact on germination and seedling growth in hybrids of sunflower used in this experiment. Also Hysun-36hybrid have more potential resistance under drought stress conditions in germination stage compared with Azargol hybrid and it could be suitable for planting at this conditions.

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# THE BEHAVIOUR OF SOME SUNFLOWER CULTIVARS TO THE MAJOR PEST AGENTS IN THE SOUTH-EASTERN AREA OF ROMANIA

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## ABSTRACT

The behavior of some sunflower cultivars against major pests were studied under natural contamination conditions at SC Sport Agra SRL, Amzacea, Dobrogea County. The pathogens which cause diseases during vegetation period were: *Sclerotinia sclerotiorum, Phomopsis helianthi (Diaporthe), Phoma macdonaldi, Alternaria helianthi* and *Sclerotium bataticola.* Climatic conditions during vegetation period in 2014, favored the manifestation of the pests in sunflower crops. One of the most dangerous parasites on the plants in Dobrogea county area was Broomrape (*Orobanche cumana)*. It was shown a significant dissemination, especially in the south and south-eastern area of Romania. In experimental plots were studied 24 sunflower hybrids. The best results for the major pest agents have showed NK ALEGO,NK MELDINI , ES EUROMIS, ES TERRAMIS, LG 55.42 CL, LG 56.61CL and FAVORIT. For the control of weeds on sunflower trial it used a specific herbicide - PULSAR- 40 (imazamox40g/l) at rate of 1,2 l/ha.
**Key words**: *sunflower,behavior major pest, control*

## INTRODUCTION

Sunflower crop is in Romania the 3rd agricultural crop after maize and wheat. In 2014, 990.000 ha were cultivated with sunflower and average production of 2129 kg / ha( NIS - Crop production for main crops in 2014). The behavior of some sunflower cultivars against major pests were studied (500mp/plot) *under* natural contamination conditions at SC Sport Agra SRL, Amzacea- Dobrogea area. The pathogens which cause diseases in vegetation periods were: *Sclerotinia sclerotiorum, Phomopsis helianthi (Diaporthe), Phoma macdonaldi and Alternaria helianthi* (Jinga et al. 2005) . One of the most dangerous parasites on plants in Dobrogea area was Broomrape (*Orobanche Cumana Wallr.).* (Pacureanu et al., 1998) It was shown a significant dissemination, especially in the south and south-eastern area of Romania. This specie has shown a significant dissemination, especially in the south and south-eastern area of Romania (Parker, 1994 , Vranceanu et al.1995. ). On sunflower crops, the losses can reach 30- 70% of the harvest. (Iliescu et al.1995, Jinga et al., 2010).

## MATERIALS AND METHODS

Experience has been placed on S.C. SPORT AGRA S.R.L. Amzacea, Constanta. The studied crops were sunflower pest. The experience was situated on a land belonging to the South Dobrogea plateau, represented by cambic chernoziom with a profile deeper than other chernozioms, a blackish-brown soil of 40-50 cm thickness, medium texture (Demeter, 2009). The content of nutrients was: mobile P index - 72; N index - 4; Humus - 3.11; K index - 200; Neutral pH - 7.2. The climate is deeply temperate continental, with an average annual temperature of 10.7-11.7°C, with a high temperature in the period 20th June to 15th August. Meteorological data are presented in

Table 1. Sowing (70 $m^2$/plot) was carried out on 6 April 2014 and observations in August. Due to heavy rains in the entire vegetation period, the attack of pathogens that cause diseases was very aggressive.

Table 1: Meteorological data 2013-2014

| Month | Temp monthly average ºC | Temp Min ºC | Temp Max ºC | Rainfall mm | Humidity % |
|---|---|---|---|---|---|
| Aug | 21.7 | 16.1 | 23.1 | 1.9 | 59.4 |
| Sept | 18.6 | 14.5 | 22.7 | 2.1 | 60.1 |
| Oct | 13.2 | 9.9 | 16.4 | 3.0 | 77.9 |
| Nov | 11.2 | 8.7 | 13.6 | 1.5 | 84.4 |
| Dec | 2.9 | -0.1 | 5.9 | 0.4 | 85.4 |
| Jan | 3.9 | 1.2 | 6.6 | 113.0 | 88.4 |
| Feb | 4.6 | 2.0 | 7.2 | 2.0 | 83.8 |
| Mar | 8.8 | 5.6 | 12.0 | 40.5 | 64.6 |
| Apr | 12.0 | 8.9 | 15.1 | 42.0 | 74.2 |
| May | 16.8 | 12.8 | 20.8 | 61.5 | 72.1 |
| June | 21.0 | 17.0 | 25.0 | 22.8 | 72.9 |
| July | 23.5 | 26.0 | 21.0 | 30.0 | 75.0 |
| Aug | 22.3 | 17.1 | 22.7 | 2.6 | 60.4 |

For the control of weeds on sunflower trial it used a specific herbicide - PULSAR- 40 (imazamox40g/l) at rate of 1,2 l/ha.

**RESULTS AND DISCUSSIONS**

Table 2: The behavior of sunflower cultivars to the major pest agents in the Amzacea plots (F%)

| Hybrids/Pest agents | *Sclerotinia sclerotiorum* | *Phomopsis helianthi* | *Phoma macdonaldi* | *Sclerotium bataticola* | *Alternaria helianthi* | *Orobanche comana* |
|---|---|---|---|---|---|---|
| NK ALEGO | 0 | 10 | 35 | 20 | 25 | 2 |
| NK NOEMA | 10 | 20 | 40 | 30 | 90 | 10 |
| NK ADAGIO | 11 | 5 | 30 | 25 | 30 | 21 |
| NK MELDIMI | 16 | 23 | 27 | 20 | 75 | 0 |
| TALENTO | 12 | 9 | 29 | 20 | 32 | 12 |
| 8 H 288 CLDM | 20 | 26 | 50 | 60 | 48 | 25 |
| 8 N 358 CLDM | 10 | 40 | 85 | 50 | 80 | 10 |
| 8 N 421 CLDM | 4 | 20 | 90 | 20 | 85 | 12 |
| 8 H 449 CLDM | 11 | 50 | 92 | 30 | 70 | 9 |
| 8 H 463 CL | 11 | 40 | 68 | 41 | 75 | 22 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ES EUROMIS CL | 12 | 21 | 70 | 32 | 88 | 0 |
| ES NOVAMIS CL | 2 | 10 | 42 | 30 | 80 | 5 |
| ES TERRAMIS CL | 7 | 2 | 72 | 21 | 75 | 0 |
| ES H 91.61 CL | 3 | 15 | 50 | 20 | 58 | 2 |
| ES BALISTIC CL | 18 | 20 | 68 | 40 | 70 | 11 |
| LG 56.33 CL | 0 | 6 | 71 | 21 | 90 | 21 |
| LG 56.63 CL | 4 | 20 | 90 | 21 | 72 | 18 |
| LG 55.42 CL | 11 | 10 | 85 | 52 | 85 | 0 |
| LG 56.61 CL | 0 | 0 | 38 | 42 | 41 | 2 |
| LUCIA CL PLUS | 2 | 30 | 82 | 32 | 80 | 18 |
| MORENA CL | 4 | 21 | 85 | 30 | 79 | 13 |
| PARAISO 1000 CL | 12 | 38 | 93 | 48 | 65 | 15 |
| SUNFLORA | 15 | 21 | 72 | 30 | 91 | 24 |
| FAVORIT | 5 | 6 | 63 | 16 | 30 | 0 |

Data presented in Table 2 it is observed that this year agriculture received numerous sediments that favored the emergence and development of the pest in sunflower crops.

The demonstration plots SC SPORT AGRA SRL Amzacea were observations made on the phytosanitary status of 24 sunflower hybrids from different companies, as shown in the attached photos.(1,2,3,4).

The pathogen *Sclerotinia sclerotiorum* has decreased in most showed hybrids attack, attack frequency being 5- 20%.

*Phomopsis helianthi* pathogen presented the higher appeals to most hybrids, between 20 and 50%. Lower frequency was found at hybrids NK ADAGIO, ES TRRAMIS and LG 5661CL .

*Phoma macdonaldi* frequency had intensity of 30-90 % .

*Sclerotium bataticola* presented the frequency between 20 and 50% in most hybrids.

*Alternaria helianthi* was also present on most hybrids, with a rate of up to 90%.

This high percentage of pathogens due to favorable weather conditions this year has particularly with very heavy rainfall and strong winds, which affected sunflower healthy crop .

Regarding parasite *Orobanche cumana* stands a few hybrids with a low attack:HK ALEGO , LG 5661 CL. Noted four hybrids without appeal, namely:NK MELDIMI, ES EURAMIS CL, ES TERRAMIS, LG 5542 CL and FAVORIT.

**CONCLUSIONS**

- In Romania, on sunflower crops in areas heavily infested with, pathogens and the broomrape especially in the south and south-eastern area of the country, as are those in Dobrogea, losses reach 30- 80% of the harvest.

- This high percentage of pathogens due to favorable weather conditions this year has particularly with very heavy rainfall and strong winds, which affected sunflower healthy crop.

- In natural inoculation on Amzacea location, good results show only 7 hybrids:

NK ALEGO, NK MELDIMI, ES EURAMIS CL, ES TERRAMIS, LG 5542 CL, LG 5661CL and FAVORIT.

- Sunflower cultivars taken under investigation show a different behavior against the attack of the parasite *Orobanche cumana*. Four hybrids without appeal, namely: NK MELDIMI, ES EURAMIS, ES TERAMIS ,LG 5542CL and. FAVORIT.

**Figure 1.** Experimental sunflower field

**Figure 2.** Hybrid 8 H 288 CLD– highest *Orobanche*. attack

**Figure 3**. Hybrid Alego -Zero *Orobanche* attack

**Figure 4**. Hybrid Favorit -Zero *Orobanche* attack

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# APPLICATION OF GEOSTATISTICS ON PHENOMIC AND PHENOTYPING DATA: AN A POSTERIORI DIAGNOSTIC OF DISEASE SPATIAL PATTERN UNDER NATURAL INFESTATION

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## ABSTRACT

Effective disease evaluation relies on a better understanding of specific interactions between host and pathogen. One problem related to soilborne diseases is the non-homogeneous nature of soilborne pathogens in terms of distribution and/or genetics that could lead to misinterpretations with respect to the presumed host resistance. The objective of this study was to analyze the *V.dahliae* distribution by looking at the spatial pattern of the sunflower Verticillium Wilt in a native system. A set of experiments were conducted from 2013 to 2015 in fields with sunflower Verticillium Wilt history. One symptomatic and one asymptomatic sunflower genotypes were introduced at specific locations (defined by spatial coordinates) to establish a grid arrangement for the disease spatial pattern evaluation. Two different sets of traits were recorded: i) reference methodology for Verticillium Wilt phenotyping (disease incidence and severity) to characterize regions of disease prevalence from 2013 to 2015, and ii) phenomic index (NDVI, passive method) to integrate senescence components in the disease evaluation in 2015. Geostatistical analyses were performed on both sets of traits and both controls to evaluate part of the micro-environment variability within the field that can interact with disease expression. The control scores were then interpolated to unsampled points through the Ordinary Kriging method. Results showed significant variation in the disease expression at field level. This confirmed that the pathogen components play a major role in the plant probability to develop the disease. No significant losses of biomass were observed, leading to the conclusion that the senescence factor did not interact with disease expression.

**Key Words :** Sunflower, *Verticillium dahliae*, geostatistics

# IMPROVING GENE-TO-PHENOTYPE PREDICTIONS WITH CROP SIMULATION MODELS: WORK IN PROGRESS FOR SUNFLOWER YIELD STABILITY UNDER WATER DEFICIT

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## ABSTRACT

The efficiency of the breeding process benefits from accurate gene-to-phenotype predictions to assign a breeding value to genotypes.Recently, hybrid modeling approaches leverage both statistical modeling (e.g. linear mixed models, LMM) and crop simulation modeling (CSM) to account for gene x environment interactions in complex traits. For example, depending on how the models are organized, (1) genomic selection frameworks can be augmented with predictors computed from crop simulation models or (2) the genotype-dependent inputs of crop simulation models can be predicted as a function of allelic composition (gene-based simulation modeling). Here, we illustrate two prediction approach es with preliminary results from the sunflower SUNRISE project, which focus on improving sunflower yield stability under water deficit. The first one is a genome-wide association study of phenotypes that were computed using field and simulated datasets on a MET of 17 locations x year x management combinations. The second is the development of a gene-based model using the SUNFLO model as crop simulation model, using data from 400 hybrids phenotyped in 5 locations.
We showed that in both approaches, statistical and numerical models complement each other to improve the prediction of non-additive gene effect and gene x environment interactions. In the association study, the characterization of water deficit at the plant level allowed to improve detection accuracy, as compared to using field measurements only. The gene-based modeling feasibility study allowed the prediction of 7 SUNFLO parameters using 48 detected SNPs.

**Key Words :** gene x environment interactions, modeling, linear mixed model, crop model, sunflower

# INVESTIGATIONS AND THE DESCRIPTION OF VIRUS DISEASES IN SUNFLOWER GROWING AREAS IN THE TRAKYA REGION OF TURKEY

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## ABSTRACT

Being an important source of vegetable oil for human consumption and the source of raw material for food industry, sunflower ( *Helianthus annuus* L.) production has been increased steadily in the World and as well as in Turkey. However there are some important virus and virus-like diseases on sunflower reducing oil seed yield and quality. Genetic disorders, herbicide injuries and downy mildew disease exhibiting systemic oak leaf pattern type mosaic, dwarfing and the sterile seed formation symptoms caused by *Plasmopara halstedii* Farlow were observed during the survey studies in 2015 in the Trakya Region of Turkey. In order to determine viruses on symptomatic sunflowers and weed hosts, 244 leaf samples were collected. For the identification of *Potato virus* Y (PVY), Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) test, for *Tobacco streak virus* (TSV) Triple Antibody Sandwich-Enzyme Linked Immunosorbent Assay (TAS-ELISA) and for the identification of *Potyvirus*'es Plate Trapped Antibody-Enzyme Linked Immunosorbent Assay (PTA-ELISA) test methods were employed. For the determination of the effect of virus infections on sunflower seed yield criteria, seeds were harvested from both infected and healthy plants separately which compared for their 1000 seed weight, hectoliter seed weight and their oil content. According to DAS-ELISA test results PVY never present in the sunflower fields of Trakya Region. Depending on symptomatic observations and the results of TAS-ELISA tests 11 out of 244 plant samples had TSV with the rate of 4.51 %. As the results of PTA-ELISA tests and the symptomatic field observations 25 of 244 plants were found infected with *Potyvirus*'es with the rate of 10.25 %. Totally 36 out of 244 plant samples revealed the presence of viruses with the rate of 14.75 % in the sunflower growing areas. This is the first report of the presence of virus infections on sunflowers in Turkey. Virus infections cause reductions of 1000 seed and hectoliter seed weights of oil seeds as the oil content was found slightly high.

**Key words**: Sunflower, *Helianthus annuus* L., PVY, TSV, *Potyvirus*

# IDENTIFICATION OF GENETIC AND MOLECULAR FACTORS INVOLVED IN SUNFLOWER PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL VARIATIONS: AN ARCHETYPE OF INTEGRATIVE SYSTEMS BIOLOGY APPROACH

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## ABSTRACT

Adaption of plants to their environment is complex and involves the genetic control of molecular, developmental and physiological processes that are largely unknown in sunflower. This complexity is even greater in hybrids where both parental genomes interact to produce heterotic phenotypes. Therefore, understanding the molecular systems involved in stress tolerance in a hybrid context is of primary importance for sunflower breeding. The SUNRISE (SUNflower Resources to Improve yield Stability in a changing Environment) project - *a French project of 8 years supported by the French National Research Agency and gathering 16 public and private partners since 2012* - aims to develop a systems biology approach to describe and model links between genes, phenotype and physiological functions at the epigenetic, transcriptomic, proteomic, enzymatic and metabolomic levels at different plant developmental stages. This integrated approach will lead to the improvement of natural genetic resources exploitation and selection of complex molecular and physiological processes involved in sunflower responses to environmental variations, especially to water constraint and in heterosis.

**Key Words :** SUNRISE, integrated approach, water contraint, hetorosis

# EXPLOITATION OF THE KNOWLEDGE ON OOMYCETE EFFECTORS TO DRIVE THE DISCOVERY OF DURABLE DISEASE RESISTANCE TO DOWNY MILDEW IN SUNFLOWER

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## ABSTRACT

*Plasmopara halstedii* is an obligate biotroph oomycete causing downy mildew disease on sunflower, *Helianthus annuus*, an economically important cultivated crop. Disease symptoms observed in fields, plant dwarfism, leaf bleaching, sporulation and production of infertile flowers, impair strongly seed yield. *P. halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different *Pl*resistance genes, not yet cloned. Number of pathotypes increased from 1 to 16 during the last 25 years in France, concomitantly with the breakdown of *Pl* resistance loci used in fields. Finding broad-spectrum *a priori* durable resistance against pathogens would open the doors to efficient, environmentally friendly and cost-effective disease control. In oomycetes, two classes of effectors are translocated into the host plant, RXLRs and CRNs, but oomycete avirulence genes described so far are RXLRs. Through high throughput genomic sequencing of 17 *P.halstedii*pathotype isolates, we selected by stringent *in silico* methods, 74 putative RXLR effectors. 33 show polymorphism with at least one pathotype whereas 41 are conserved in sequence among the 17 pathotypes. Analysing the pathotype effector polymorphism in regard to the content in *Pl* resistant genes of sunflower lines should help us to identify candidates for pathogen avirulence genes. Triggering of defense reactions (Hypersensitive Response) through their transient expression in sunflower lines carrying known resistance genes will be used to validate them. Subcellular localization experiments of selected candidate effectors fused to GFP should give hints to their function in the plant cell. In addition, polymorphic effectors will be used to design molecular markers for rapid pathotype identification. Thirty conserved effectors corresponding to highly expressed genes upon sunflower infection are suspected to be essential genes for the pathogen. They have been cloned and are tested by agroinfiltration on various resistance sources of *H. Annuus* and some of them induce plant cell death. Co-segregation of resistance with cell death activity caused by the effector will have to be tested on segregating populations. If true, these effectors should accelerate the identification, the functional characterization and the mapping of broad-spectrum sunflower resistances potentially sustainable.

**Key Words :** downy mildew, disease resistance, oomycete effectors

# SUNFLOWER BREEDING STRATEGY FOR RESISTANCE TO DOWNY MILDEW DISEASE IN INDIA

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## ABSTRACT

Sunflower downy mildew disease is caused by *Plsamopara halstedii* (farlow) and was first reported in 1986 in Marathwada region of Maharashtra state (India) on well adapted cultivar Morden with 1-36 % intensity. The occurrence of disease was reported in other states of India *viz*., Karnataka, Andhra Pradesh and Punjab. For effective screening of the sunflower germplasm, varieties and hybrid for the downy mildew disease, under the controlled condition the sick plot technique was developed at Oilseeds Research Station Latur in 1988. Hence, the centre has been identified as facilitator for the screening of the advance material evaluated under All India Coordinated Research Project (AICRP) on Sunflower for downy mildew at National level. The results of sick plot revealed that the disease reduces sunflower seed yield up to 89 % and negatively affects the other traits. The race identification studies of Indian isolate of *Plsamopara halstedii* revealed that it belongs to race-1 (European race). Breeding for downy mildew resistance is one of the major goals in sunflower breeding programme in India. The research work carried out since1988 to 2015 at the centrer under AICRP on sunflower screened the advance breeding material in both field and sick plot condition. Till date 5408 sunflower accessions against downy mildew were evaluated and reported 1075 disease free having high level of resistance to the pathogen. This resulted into the release of 15 sunflower hybrids and populations at national level. The identified resistance sources have been effectively utilized in the introgression of the resistant genes from identified sources for the improvement of parental lines. The centre has identified 14 parental lines (8 CMS, 6 restorer lines) possessing downy mildew resistant (Pl genes). The center has released three state level hybrids LDMRSH-1, LDMRSH-3 and LSFH-35 and LSFH-171 at national level for commercial cultivation.

## INTRODUCTION

In India different diseases are the main limiting factor in the production of sunflower (*Helianthus annuus* L.) and they cause poor realization of genetic yield potential of sunflower hybrid. Downy mildew (DM) is an economically significant disease. It is caused by the fungus *Plsamopara halstedii (Farl.).* Downy mildew is widespread in all sunflower growing countries with the exception of Australia.With regards to India, during early 1980's Ram Nath *et al* (1981) detected oospores of *Plsamopara halstedii* on sunflower seeds imported from Bulgaria. Mayee and Patil (1986) reported its occurrence in Marathwada region of Maharashtra state on *cv* Modern with 10.0 percent intensity for first time. The DM occurrence was immediately brought to the notice of Oilseeds Researchers in the Annual *kharif* workshop held at Dr. Punjabrao Deshmukh Agrilculture University, Akola of Maharashtra State (Anonymous, 1985). Similarly suggestions to restrict the disease with wide publicity were given through regional and national news papers for keeping watch on disease spread. (The Hindu, January 1$^{st}$ 1986, Indian Express, April, 23$^{rd}$ 1985). A Committee was constituted to go in the details of DM occurrence and the committee felt that the disease has been introduced through infected seeds and probable failures of quarantine detection has resulted in the introduction of disease. Recently a survey was conducted during 1995-96 to find out the present status of the disease in Marathwada region of Maharashtra State in India. Sixty fields in six districts were visited and out of those, 22 fields (36.67 %) had DM incidence with

varying intensity ranging from 1 to 30.00 per cent (Shrishikar, 1995). The cv Modern was highly infected by the disease as compared to hybrids.

The extent of damage depends on infection type i.e. whether it is primary (systemic) or secondary infection, while primary or systemic infection causes significant yield reductions. Primary infection is effected during seed germination in the soil and the emergence of sunflower seedling. It may caused by Fungus mycelium or oospores present on infected seeds or by oospores present in the infected soil in to which healthy seeds were sown. No matter if primary infection starts from seeds or soil, the causes of disease development in infected plants is identical.

The fungus develops in unison with the development of young plants. It penetrates the root, stem cotyledons and reaches the meristematic tissues at the top of young plants. The fungus developments inside the infected plants intercellular in all plant parts, pervading the young tissues and depriving the infected plants of assimilates and water. This is why infected plants lag behind healthy ones in growth and development. This way of fungus expansion inside the plant tissue is called a systemic infection. It begins with the infection of the germ and ends with the infection of the head and seeds. The fungus penetrates all parts of the seed (Husk, endosperm and germ) which then produces a new infected seedling. Infected plants in addition to having stunted growth i. e. short internodes are chlorotic and with a platform head which gives a smaller yield than the normal head. On the infected plant parts, the roots, cotyledons, the stem and especially the leaves, there occurs abundant white mycelium, which is typical for this disease. The mycelium occurs also on the reverse side of the leaves and it contains the vegetative organs of the fungus conidiophores and conidia (Zoosporangia). On the upper side of the leaf there occurs clorotic spots. Infected plants collapse and remain in the field after harvest. Measures of protection against Downy mildew include cultivation practices, chemical measures and the use of resistant hybrids.

The most effective chemical measures of Downy Mildew control is seed treatment with metalaxyl based preparations. These measures protect the sunflower crop at the time of the primary infections i.e. early stage of development of sunflower. Breeding for downy mildew resistance is one of the major goals in sunflower breeding programme in India. The research work carried out since1988 to 2015 at the Latur centrer under AICRP on sunflower screened the advance breeding material in both field and sick plot condition. Use of genetically resistant hybrids is definitely the most effective way of controlling Downy mildew in sunflower. Therefore research work set up with the objective of developing sunflower genotype genetically resistant to dominant race of Downy mildew in India gained prime importance in sunflower breeding programme.

## MATERIAL AND METHODS

To determine the variability and level of resistance available in cultivated sunflower (1988-2015) 5408 accessions which include CMS, inbreds, restorer, open pollinated varieties, germplasm lines and hybrids collected from NBPGR, IIOR, Hyderabad and different AICRP (All India Coordinated Research Project) sunflower centers in India were evaluated in field along with Downy mildew sick plot unique developed at Oilseeds Research Station, Latur, Maharashtra.

### Inoculation Technique

Many inoculation techniques viz. soil inoculation, seed inoculation, radical inoculation, foliar spray method, whole seedling immersion and disc method have been described for artificial screening of sunflower genotypes against DM disease. Patil *et al* (1993) have compared all these methods to find out effective technique for artificial screening of sunflower genotypes against DM under controlled conditions. The methodology for seed inoculation and Radical inoculation technique is given below

1. **Seed inoculation:** Seed of cv Modern were soaked in water for five hrs and kept in rolled towel paper. Next day these seeds were immersed in zoosporangial suspension (2.5 x $10^4$ propagules/ml of distilled water) for five hrs and sown in pots).
2. **Radicle inoculation:** In this method the germinated seeds of cv Morden were inoculated with zoosporangial suspension, when radical was 4 to 5 mm long by spraying of sporangial suspension @ (2.5 x $10^4$ propagules/ml of distilled water).

## RESULT AND DISCUSSIONS

From the table 1 it can be conclude that the radical inoculation method is the best method for screening sunflower genotypes against DM. Similarly Wehtje and Zimmer (1978) have reported that in sunflower seedling infected with P. halstedii, the zoospore encystment and infections occurred primarily, within or adjacent to zone of elongation of radical and up to 1000 infection sites / mm of root length were observed.

Table : 1 Incidence of Downy mildew as influenced by different inoculation techniques (1986-89)

| S.No | Method | Percent disease incidence | | | Mean |
|---|---|---|---|---|---|
| | | 1986-87 | 1987-88` | 1988-89 | |
| 1 | Soil inoculation | 52 | 55 | 42 | 49.66 |
| 2 | Seed Inoculation | 52 | 56 | 60 | 56.00 |
| 3 | Radicle inoculation | 100 | 95 | 90 | 95.00 |
| 4 | Foliage Spray | 60 | 58 | 57 | 58.33 |
| 5 | Whole seedling immersion | | | | |
| | a) Cotyledon method | 65 | 64 | 69 | 66.00 |
| | b) First two leaf stage | 64 | 64 | 69 | 63.66 |
| 6 | Disc method | * | * | * | |

- Only local lesions were observed

**Race situation and sources of resistance**

The fungus completes its sexual life cycle annually, affording maximum opportunity for recombination of genes responsible virulence and / or other pathogenic characters. Initially two races of the fungus were differentiated, race- 1, originally referred to as the European race and race -2, known as Red River race, referring to the Red River vally area of North Americ. However, presently a total eight races are known worldwide (Viranyi, 1990). With regards to India, Patil and Mayee (1990) conducted the studies to find out the race situation of India isolate of *Plasmospara halstedii* and study revealed that (Table 2) it belongs to race - 1. The reaction to different isolates observed on various 'R' lines which resulted in identification of five resistant restorer lines in India.

Table : 2 Reaction of *Plasmospara halstedii* Indian isolate to Downy mildew sunflower host differentials

| SN | Differential Line | Percent disease incidence | | | Known reaction to race | | | Reaction to Indian isolate |
|---|---|---|---|---|---|---|---|---|
| | | 1986-87 | 1987-88 | 1988-89 | 1 | 2 | 3 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | RHA-272 | 2.00 | 0.56 | 0.90 | R | R | S | R |
| 2 | RHA-273 | 0.80 | 0.83 | 0.43 | R | R | S | R |
| 3 | RHA-274 | 0.00 | 0.00 | 0.00 | R | R | S | R |
| 4 | RHA-801 | 0.00 | 0.00 | 0.00 | R | R | S | R |
| 5 | RHA-265 | 0.66 | 0.56 | 0.83 | R | S | S | R |
| 6 | Progress | 58.66 | 63.33 | 60.00 | S | S | R | S |

R = Resistant; S = Susceptible

The progress cultivar is known to resistant to race -3, but it is susceptible to Indian isolate, hence the Indian isolate does not belong to race -3. The cultivar RHA-265 is resistant to race -1 and susceptible to race -2 and 3. The same cultivar is also resistant to Indian isolate; hence the Indian isolate could be race -1 category. A regular field screening programme for sunflower lines is being conducted at Oilseeds Research Station, Latur (MS) to find out the reaction of sunflower lines against DM under sick soil condition, since from 1988. Two DM resistant hybrids *viz*., LDMRSH-1 (CMS-338-A x MRHA-2) and LDMRSH-3 (CMS-207-A x MRHA-1), LSFH -35 (234 A x RHA-1-1), LSFH-171 (CMA-17A x RHA-1-1) developed at Oilseeds Research Station, Latur have been released.

**Disease management**

The DM disease is seed, soil and air borne in nature, it is necessary to adopt various control strategies like regulatory measures, cultural management, seed treatment and use of resistant varieties etc. to combat the disease under field condition.

**Host resistance**

This includes use of resistant varieties (LDMRSH-1 and LDMRSH-3) to combat DM problem (Patil et al 1992). Use of hybrid varieties should be encouraged sine they are found tolerant compared to population. Similarly at Oilseeds Research Station, Latur many DM resistant hybrids have been identified through screening in DM plot and based on yield potential and DM resistance, ICAR has released such hybrids for the commercial cultivation (Shrishikar, 2005) (Table 4 ).

Table 4: List of sunflower Downy mildew resistant / tolerant hybrid s identified by ICAR varietal release committee (2002- 15)

| Name of sunflower hybrids / variety | Year | Remarks |
|---|---|---|
| Sungene – 8 | 1996 | This variety has been released during AICRP workshop held at JNKKV, Jabalpur in April 1996 |
| LS-11 | 1998 | This variety released by varietal identification committee meeting held at TNAU, Coimbtore in April 1998 |
| MSFH-47 | 2000 | This hybrid was highly resistant to DM and it was released by ICAR varietal identification committee meeting at PAU, Ludhiana in April 2000 |

| | | |
|---|---|---|
| Pro-009 (Prosun-09) | 2003 | This hybrid has been releases during AICRP sunflower workshop held at TNAU, Coimbtore |
| SH-416 | 2003 | This hybrid has been releases during AICRP sunflower workshop held at TNAU, Coimbtore |
| DRSF-108 | 2003 | This hybrid has been releases during AICRP sunflower workshop held at TNAU, Coimbtore |
| PCSH – 243 | 2004 | This hybrid has released during AICRP sunflower workshop held at ANGRU, Hyderabad |
| RPO-011 | 2004 | This hybrid has released during AICRP sunflower workshop held at ANGRU, Hyderabad |
| SCH-35 | 2004 | Released by M. S. State, ORS, Latur Hybrid |
| XF-4132 | 2005 | This hybrid has been released during AICRP sunflower workshop held at H. P. Krishi Vishwa Vidhyala, Palampur |
| PAC-334 | 2008 | This hybrid has been released during Annual Group meeting held at GAU, Junagarh on 21-23 May 2009` |
| LSFH-171 | 2012 | This hybrid has been released during Annual Group meeting held at UAS, Bangaluru on 27-29 April 2012 |

**Chemical control**

Seed treatment with fungicide like Apron 35 S. D. (Metalaxy fungicide) found very effective for the control of DM disease. The efficiency of Apron 35 S.D. fungicide and they reported that the fungicide is quite effective in reducing DM incidence under field conditions when used @ 6 g / kg of seed. However, a new formulation Apron XL 35 ES- @ 3 ml / kg as seed dresser has also been recommended (Shrishikar, 2005) (Table 3).

Table 3: Management of sunflower DM disease through Apron XL 35 ES fungicide under sick plot condition 2002-04 (Pooled)

| SN | Details | DM incidence (%) | Yield kg/ha | BC ratio |
|---|---|---|---|---|
| **1** | Apron1 ml / kg | 20.40 | 726 | 1.81 |
| **2** | Apron 2 ml / kg | 14.86 | 861 | 2.14 |
| **3** | Apron 3 ml / kg | 5.46 | 1106 | 2.74 |
| **4** | Apron 6 ml / kg | 8.56 | 1043 | 2.57 |
| **5** | Control | 85.5 | 263 | 0.65 |
| | **SE ±** | 1.1 | 106 | |
| | **CD at 5 %** | 3.4 | 326 | |

**CONCLUSION**

The race identification studies of India isolate of *Plasmopara halstedii* confirms that it belongs to race -1 (European race). The multiyear work carried out at ORS, Latur centre under All India Coordinated Research Project on sunflower screened 5408 lines and identified 1075 disease free entries with high level of resistance to DM pathogen. This resulted into the release of 15 sunflower hybrids and populations at national level. The identified resistance sources have been effectively utilized in the introgression of the resistant genes from identified sources for the improvement of parental lines. The centre has identified 14 parental lines (8 CMS, 6 restorer lines) possessing downy mildew resistant (Pl genes). The center has released three state level hybrids LDMRSH-1, LDMRSH-3 and LSFH-35 and LSFH-171 at national level for commercial cultivation.

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# THE BEHAVIOR OF SUNFLOWER HYBRIDS IN DIFFERENT ENVIRONMENTAL CONDITIONS IN ROMANIA

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## ABSTRACT

Sunflower breeders, working for a model (idiotype) of sunflower, must to know the main characteristics of the environment for which they are developing the hybrids, starting from soil type, potential growing season length, mean, minimum and maximum temperatures (per month) and the amount and distribution of rainfall, during the year. In the practical selection, which is part of the production of hybrids with high potential of productivity, as well as high adaptive potential, a strong influence belongs to the adaptive reactions to the ecological environment they are located in. The dry periods are more frequently, with negative effect on yield, including sunflower. We studied a set of 10 sunflower hybrids, in two years (2014 and 2105), in two locations, situated in different areas in Romania: Braila (eastern Romania and Fundulea (south Romania). The hybrids have been cultivated in three randomized replications. Comparing the two years, 2014 and 2015, regarding the air temperature and the amount of rainfall, in sunflower vegetation period generally, year 2015 was more dry, specially in Braila. The amount of rainfall was quite high, in Fundulea location, in 2014 year. Taking into consideration these data, the results regarding the seed yield for the ten hybrids, are showing that in Braila location it was registered a low seed yield comparing with Fundulea, in 2014 year. In 2015 year the highest seed yield was released by the hybrids, in Braila location. The hybrids Fundulea 708 and PR64LE20 had a good behavior, regarding the seed yield. The oil content, for all hybrids, was very good, in both years, in Braila location, the soil and climatic conditions in this location, being favorable for this characteristic. Regarding plant height, in both location and both years, the taller hybrids released the highest seed yield.

**Key Words :** sunflower, environmental conditions, hybrids, seed yield, oil content

# HISTORY AND PRESENT STATE OF DOWNY MILDEW IN ARGENTINA

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## ABSTRACT

Until 1998, races of *Plasmopara halstedii* (Farl.) Berl. *et* de Toni present in Argentina were 300 and 330, and almost all the sunflower hybrids sown in the country were resistant by the introgression of *Pl*2 resistant gene. Since that year resistance conferred by Pl2 was broken and races 710, 730 and 770 were determined. Resistance genes to these new races were introgressed from public lines from USA and Argentinean populations (*Pl5, Pl6, Pl7, Pl8, Pl15, Pl17, Pl18* and *PlArg*). Seed treatment with metalaxyl has been also widely used in different sunflower areas, but strains tolerant to this fungicide were found in all these races. Since 2013, downy mildew has been found in hybrids containing *Pl*15 gene, indicating the presence of a new race which is not possible to be classified by the international set of differential lines. Sustainable management of this disease should be based on reducing the selection pressure over the pathogen by combining practices as the introgression of several resistance genes simultaneously, using different active ingredients for seed treatment, crop rotations and avoiding contaminated seed exchange.

**Keywords:** metalaxyl tolerance; *Plasmopara halstedii;* new races; differential inbred line; *Pl* 15 resistant gene.

## INTRODUCTION

Sunflower (Helianthus annuus L.) downey mildew (DM) caused by the oomycete *Plasmopara halstedii* (Farl.) Berl. & de Toni is one of the major diseases of this crop worldwide. It is potentially destructive when early infections occur through the roots causing damping-off and dwarfism. Usually, *P. halstedii* can generate variants of pathogenicity (pathotypes) with high frequency. Seed exchange between countries and / or production regions may favor the spread of these new pathotypes. Currently, there are at least 36 pathotypes of *P. halstedii* worldwide, but the number of races is increasing rapidly (Virányi and Spring, 2011). Furthermore, during the 80´s and 90´s many cases of *P. halstedii* tolerant to metalaxil were report from Spain, Hungary (Oros and Virányi, 1984), Turkey (Delen *et al*., 1985), France (Lafon *et al*., 1996) and USA (Gulya *et al*., 2000) among others.

Until 1998, the races of *P. halstedii* present in Argentina were 300 and 330 (Bazzalo, *et al*., 1996), and almost all the sunflower hybrids were resistant by the introgressed *Pl*2 resistant gene. Since that year, resistance conferred by *Pl*2 was broken by new races (710, 730 and 770 races) (ASAGIR, 2003). Resistance genes to these races were introgressed from public lines from USA and Argentinian populations (*Pl*5, *Pl*6, *Pl*7, *Pl*8, *Pl*15, *Pl*17, *Pl*18 and *Pl*Arg) (Bertero de Romano, *et al*. 2010). Cultivars with genetic resistance and/or seed-coated with metalaxyl or metalaxyl–M (mefenoxam) are strategies widely used for disease control in Argentina. Crop rotation with low presence of sunflower, a delay in sowing dates in high soil density (high clay content and no tillage) can also contribute to disease control. However, between 2012 and 2015 the prevalence and

intensity of DM has increased in different production regions of Argentina, indicating changes in pathogenicity of *P. halstedii*. The aim of this study was to identify new pathotypes of *P. halstedii* associated with the occurrence of epiphytotics of DM in Argentina. With this objective we have identified DM epiphytotic in different production regions of Argentina and then collected *P. halstedii* inoculum (sporangia) to determinate race and / or tolerance to metalaxyl.

## MATERIALS AND METHODS

Between 2012 and 2015 seasons the presence of DM epiphytotics (> 5% incidence) were identified in different production regions of Argentina. The geographical location and the implemented strategies for DM control (genetic resistance and/or seed-coated with metalaxyl) were recorded for each case. Isolates of *P. halstedii* were collected. For inoculum multiplication, susceptible sunflower genotypes (without *Pl* genes or only *Pl*2; Paraiso 20®, Nidera Argentina; Cauquen®, El Cencerro, Argentina; HA 89 public line) were inoculated with each isolate using a protocol adapted from Viranyi and Gulya (1995). The resulting inoculum was briefly stored at -20ºC.

Twenty-eight isolates were selected and used to carry out the tests of tolerance to metalaxyl and/or race determination. Seeds of susceptible genotypes were treated with metalaxyl (46 mg per seed) to determine the tolerance of each isolate. A control without metalaxyl was included to determinate de level of tolerance. A randomized complete block design was used with two or three repetitions. Each experimental unit consisted of ten seeds planted in speedlings in a soil:perlite (1:1) substrate. The inoculation was performed according to the protocol of Viranyi and Gulya (1995). For the disease evaluation (incidence: plants with signs of DM per total plants), susceptibility was considered when sporulation on cotyledons and the first true leaves became evident to the naked eye. Occasionally, damping-off could be seen among the inoculated seedlings. Data was analyzed with ANOVA and LSD Fischer for disease incidence media comparison. For the race determination the sets of sunflower differential lines proposed by Tourvieille de Labrouhe, *et al*. (2000) were used. Seeds inoculation, seedling cultivation and disease evaluation were carried out as described above.

## RESULT AND DISCUSSION

DM epiphytotics were identified in the three sunflower production regions of Argentina (Figure 1). North Santa Fé province and south Buenos Aires province were the regions with major number of cases (6 and 20 cases, respectively) between 2012 and 2015 crop seasons. In north Santa Fé, five *P. halstedii* isolates were tolerant to metalaxyl (Barro Pazos, Ceibal, Malabrigo, Reconquista and Villa Ocampo). These isolates were identified as 710, 730 and 770 races (Figure 1) (Bazallo 2014, Bazzallo and Piubello, 2015). Also, the presence of 713 race was detected near Santa Fé city (Figure 1; Table 1). It is the first registry in Argentina of *Pl*13 and *Pl*14 break (Table 1).

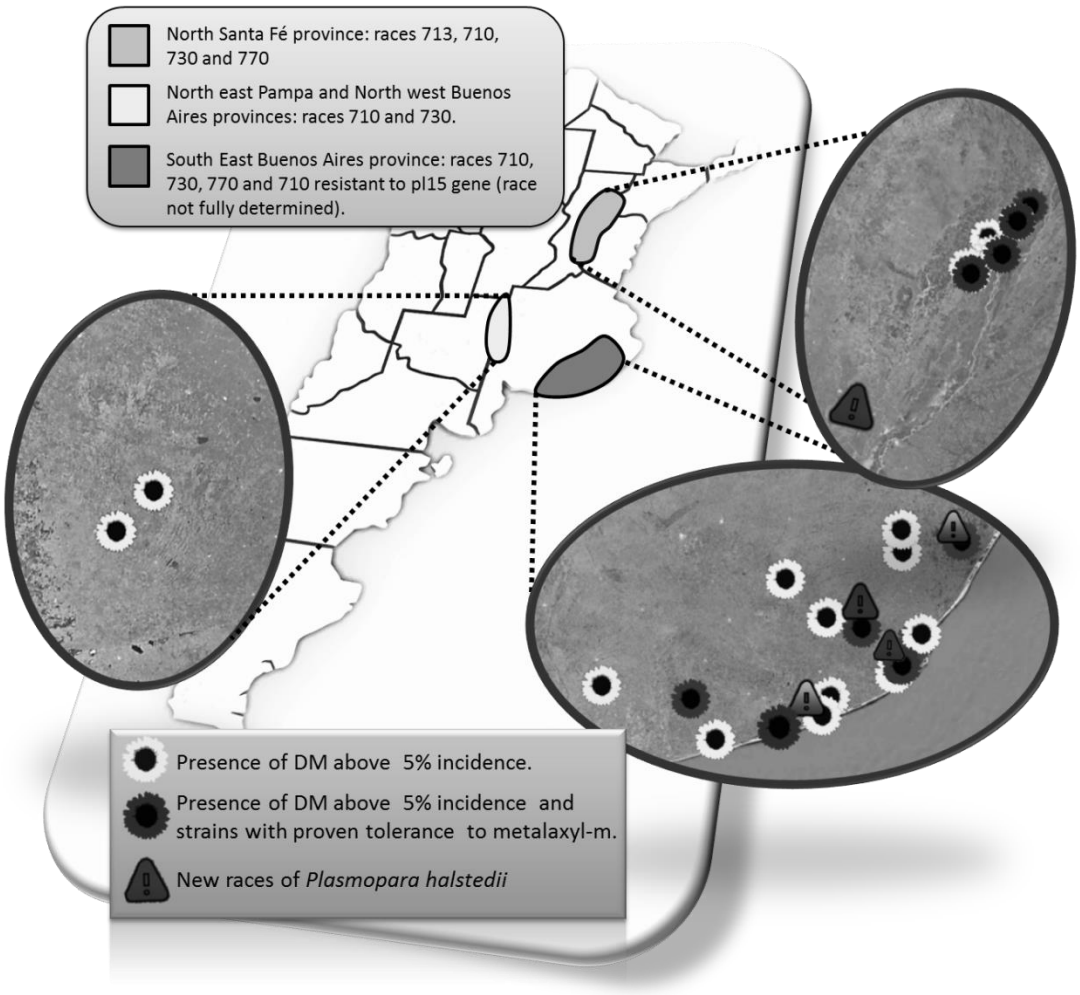


Figure 1. Location of downy mildew epiphytotics (more than 5% of incidence) caused by *P. halstedii* in Argentina between 2012 and 2015. Torelant isolates to metalaxyl and/or new races are discriminated.

In south of Buenos Aires province, twelve *P. halstedii* isolates were corroborated as tolerant to metalaxyl (Balcarce, Mar del Plata, Otamendi, Tres Arroyos among others) and were identified as 710, 730 and 770 races (Figure 1) (Erreguerena *et al*., 2013; Bazallo 2014, Bazzallo and Piubello, 2015). In this region, in 2014-2015 seasons it was found that some hybrids with resistant (*Pl*15 gene) to widespread Argentinian races (710, 730 y 770) were affected by DM. In these cases, the *P. halstedii* isolates were characterized as 710 race, although these variants compared with ordinary 710 races broke *Pl15* resistance gene (Table 1).

Table 1: New *P. halstedii* races in Argentina characterized by their reaction of sunflower differential line suggested by Tourvieille de Labrouhe, *et al.* (2000) and by an inbred line with *Pl*15 resistance gen.

| | | | | | New races in Argentina | |
|---|---|---|---|---|---|---|
| Diferencial set | | Line | *Pl* Gen | Race 710 ordinary variant (Argentina) | Race 710 variant not fully discriminated | Race 713 |
| SET ONE | D-1 | HA89 | | S | S | S |
| | D-2 | RHA265 | 1 | S | S | S |
| | D-3 | RHA274 | 2 | S | S | S |
| | | | | | | |
| SET TWO | D-4 | PMI-3 | 4 | S | S | S |
| | D-5 | PM-17 | 5 | R | R | R |
| | D-6 | 803-1 | 803 | R | R | R |
| | | | | | | |
| SET THREE | D-7 | HAR-4 | 14 | R | R | **S** |
| | D-8 | HAR-5 | 13 | R | R | **S** |
| | D-9 | HA-335 | 6 | R | R | R |
| | | | | | | |
| ***Inbred line with Pl 15*** | | NI-Pl15 | 15 | R | **S** | R |

The present context of sunflower production in Argentina shows a growing dynamics towards to the generation of pathogenic variants of *P. halstedii* than the historically observed. In recent years there has been determined the tolerance to metalaxyl and the appearance of at least two new races (713 and 710 race that break the *Pl*15 resistance). *Plasmopara halstedii* pathotypes with tolerance to metalaxyl are distributed mostly in the regions of sunflower production, except for center region (east of La Pampa and west of Buenos Aires provinces). The loss of effectiveness of metalaxyl as a seed control requires its replacement with other molecules. The *Pl15* break by a new race exposes some of the Argentinian commercial hybrids to DM increasing the risk of an epyphitotic occurrence in the southern region of Buenos Aires province. For an accurate discrimination of this new race in relation to 710 ordinary race is required to expand the differential set of inbred lines suggested by Tourvieille of Labrouhe, et al. (2000). In this context, the *Pl6* and *Pl8* genes widely introgressed in Argentinian hybrids remains efficient for controlling DM. Is important to implement molecular techniques for *P. halstedii* identification in seed and avoid the incorporation of the pathogen or its pathotypes in the crop regions free of DM. Sustainable management of disease should be based on reducing the selection pressure over the pathogen by combining practices as the simultaneously introgression of several resistance genes, using alternative active ingredients for seed treatment, crop rotations and avoiding early sowing date and contaminated seed exchange.

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# A REVIEW ON THE SEED-BORNE MICROFUNGI OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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## ABSTRACT

Sunflower (*Helianthus annuus* L.), is one of the major oilseed crop grown for edible oil in all over the world and is prone to seed-borne fungi during harvesting and storage. These saprophytic and parasitic fungi may affect the seed quantity and quality. They can cause reduction of the amount of fatty acid in the seed and/or the formation of mycotoxins during storage. Fungal species can vary depending on storage conditions. In this review, the researches carried out on seed-borne microfungi of sunflower were evaluated. In studies on seed-borne fungi of sunflower, *Aspergillus, Penicillium, Fusarium, Alternaria, Rhizopus, Mucor, Curvularia, Cladosporium, Trichoderma, Macrophominia, Emericella*, *Stemphylium*, *Chatemium* and *Phoma* were reported as the genera most commonly found. And, the most frequently isolated species from seeds were *Aspergillus flavus, Aspergillus niger, Alternaria alternata, Rhizopus stolonifer, Aspergillus fumigatus, Aspergillus ochraceus, Curvularia lunata, Fusarium solani, Fusarium moniliforme, Aspergillus nidulans, Mucor mucedo, Cladosporium cladosporioides*and *Penicillium chrysogenum*. In addition, the presence of mycotoxins such as aflatoxins (B1, B2, G1 and G2), sterigmatocystin, ochratoxin A, zearalenone, T2-toxin, diacetoxyscirpenol, alternariol and alternariol monomethyl ether were recorded from various researches.

**Key Words :** sunflower, seed, seed-borne microfungi

# EPIPHYTOTIC DISEASE OF SUNFLOWER STEM CANKER IN ARGENTINA

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## ABSTRACT

The canker on sunflower stem was diagnosed in America, Europe and Australia (Marisevic & Gulya, 1992; Thompson *et al*., 2011). Although it was detected in Argentina the disease was sporadic (1985) while in Uruguay, from 2003, has been found as a serious disease (Huguet 2006, Stewart 2005). In the 2015 and 2016 growing seasons high-incidence outbreaks of the disease have been important in the Northwestern of La Pampa Province, Southern of Córdoba Province and Northeastern of Buenos Aires Province. The symptoms on sunflower stems presented pale brown cankers developed around petioles insertions. The leaf blades in connection with cankers shown V-shaped necrosis. The leaves laterally disposed above the canker presented intervein necrosis. On the bases of capitula brown rotten areas were observed affecting receptacles and even achenes (discoloured seeds). The receptacles presented necrosed bracts, expanded V-shaped necrosis pointing to and even involving the peduncles. The degree of susceptibility was recorded on 36 commercial sunflower hybrids under naturally conditions. The incidence of stem canker ranged from 1.25 % to 33.75 %. In some cases sunflower fields presented rot incidence of 100 % on capitula (R8 phenological stage). Yield losses are still under evaluation. From the symptoms described on sunflower stems and capitula several fungal isolates were obtained according to the methodology described by Muntañola et al. (1981, 1985). Isolates were morphologically determined as *Phomopsis* cf. *helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov. *Nova Hedwigia* 34: 433 (1981). Molecular studies in connection with isolates from stems (and capitula) are being carried out by Dr. Sue Thompson (DEEDI, Australia). Koch's postulates were completed on healthy sunflower plants. The isolates have presented pycnidia semi-immersed, dark brown, separate or confluent, subglobose to ampulliform, 480-630 x 440-530 μm, with exuding pale yellow drop-like slime. Conidiogenous cells were cylindrical, gradually tapering into necks, hyalines, 9.6- 15.4 x 1.4-1.9 μm. Alpha conidia were not observed. Beta conidia were filiform, sigmoid, hamate, 17-32 x 0.96 μm. *Phomopsis* cf. *helianthi* was also isolated from stem's cankers on the common weed *Helianthus petiolaris* Nutt. Other isolates are under evaluation on other weeds in order to detect potential pathogen hosts. The results obtained could reflect an expanding outbreak of the sunflower stem canker in Argentina.

**Keywords:** sunflower, phomopsis, diseases, stem canker, hybrids

## INTRODUCTION

The canker on sunflower stem was diagnosed in America, Europe and Australia (Marisevic & Gulya, 1992; Thompson *et al*., 2011). Although it was detected in Argentina the disease was sporadic (1985) while in Uruguay, from 2003, has been found as a serious disease (Huguet 2006, Stewart 2005). In the 2015 and 2016 growing seasons high-incidence outbreaks of the disease have been important in the Northwestern of La Pampa Province, Southern of Córdoba Province and

Northeastern of Buenos Aires Province. Symptoms were observed on leaves, stems and capitulas. There are no antecedents of epiphytotic disease of sunflower stem canker in this region.
The objectives of study were to describe the symptoms in plants and determine a causal agent of disease, determine alternative hosts of sunflower stem canker, and characterize the health behavior in sunflower hybrids in this region.

## MATERIALS AND METHODS

In northern of La Pampa Province (S 35° 34' 43.4" W 63° 41' 19.75"), 36 sunflower hybrids in 3 trials grouped according to their characteristics were seeded: a) resistant to imidazolinone (IMI resistant, 18 hybrids) b) Normal and high oleic (10 normal hybrids and 2 high oleic hybrids), c) Confectioner (6 hybrid). The plots consisted of three rows with row spacing of 0.52 m and 9 m long. A density of 57,000 plants $ha^{-1}$ was used in test a and b. In trial c, density was 40,000 plants $ha^{-1}$ with equal row spacing. All cultivars were planted at 23/10/2015 in cero tillage on soybean predecessor, in a sandy loam soil. In each trial design it was done in randomized blocks with 4 repetitions.
Health behavior was evaluated on each experimental unit in 20 plants under natural infection. The incidence of plants with canker on the stem in the phenological state of R7 (Schneiter an Miller 1981) was determined.
From the symptoms on sunflower stems and capitula several fungal isolates were obtained. Fungal material was isolated and established in pure culture on Malt Extract Agar prepared according to Booth (1971) (Muntañola-Cvetković et al., 1981, 1985) using Malt Extract Oxoid LP 0039; pH = 6 before sterilization without any hydroxide addition. Vancomycin (250 ppm) was incorporated into the isolating media in order to suppress bacterial development. Incubation was carried out at 26-28 ºC under 16 h UV light (345-400 nm)/8 h obscurity cycles. Sporulation starts after ten incubation days. Measures were taken from two weeks cultures picking up pycnidia holding pale yellow slime (5 pycnidia, 10 conidiogenous cells, 10 conidia). Specimens were mounted on cotton blue 0.1 % w/v in lactic acid 85 % w/w and Shear's mounting fluid.

## RESULT AND DISCUSSION

The symptoms on sunflower stems presented pale brown cankers developed around petioles insertions. The leaf blades in connection with cankers shown V-shaped necrosis. The leaves laterally disposed above the canker presented intervein necrosis. On the bases of capitula brown rotten areas were observed affecting receptacles and even achenes (discoloured seeds). The receptacles presented necrosed bracts, expanded V-shaped necrosis pointing to and even involving the peduncles.
The incidence of stem canker ranged from 1.25 to 33,75 % for sunflower IMI resistant hybrids. The normal and high oleic hybrids ranged from 6.25 to 33.75 %, while confectioner hybrids have presented incidence from 13,75 to 30 %.
In some cases sunflower fields of farmers presented rot incidence of 100 % on capitula (R8 phenological stage). Yield losses are still under evaluation.
From the symptoms described on sunflower stems and capitula several fungal isolates were obtained according to the methodology described by Muntañola et al. (1981, 1985). Isolates were morphologically determined as *Phomopsis* cf. *helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov. *Nova Hedwigia* 34: 433 (1981). Molecular studies in connection with isolates from stems (and capitula) are being carried out by Dr. Sue Thompson (DEEDI, Australia). Koch's postulates were completed on healthy sunflower plants. The isolates have presented pycnidia semi-immersed, dark brown, separate or confluent, subglobose to ampulliform, 480-630 x 440-530 μm, with exuding pale yellow drop-like slime. Conidiogenous cells were cylindrical, gradually tapering into necks, hyalines, 9.6- 15.4 x 1.4-1.9 μm. Alpha conidia were not observed. Beta conidia were filiform,

sigmoid, hamate, 17-32 x 0.96 μm. *Phomopsis* cf. *helianthi* was also isolated from stem's cankers on the common weed *Helianthus petiolaris* Nutt.
Other isolates are under evaluation on other weeds in order to detect potential pathogen hosts.
The results obtained could reflect an expanding outbreak of the sunflower stem canker in Argentina.

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# INVESTIGATIONS AND THE DESCRIPTION OF VIRUS DISEASES IN SUNFLOWER GROWING AREAS IN THE TRAKYA REGION OF TURKEY

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## ABSTRACT

Being an important source of vegetable oil for human consumption and the source of raw material for food industry, sunflower ( *Helianthus annuus* L.) production has been increased steadily in the World and as well as in Turkey. Edirne, Kırklareli and Tekirdağ provinces in the Trakya region have been important sunflower growing areas in Turkey. In order to determine virus diseases reducing oil seed yield and quality survey studies were conducted in two different periods during 2015 growing season. In order to determine viruses on symptomatic sunflowers and weed hosts, 244 leaf samples were collected. For the identification of *Potato virus* Y (PVY), Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) test, for *Tobacco streak virus* (TSV) Triple Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (TAS-ELISA) and for the identification of Potyvirus'es Plate Trapped Antibody-Enzyme Linked Immuno Sorbent Assay (PTA-ELISA) test methods were employed. For the determination of the effect of virus infections on sunflower seed yield criteria, seeds were harvested from both infected and healthy plants separately which compared for their 1000 seed weight, hectoliter seed weight and their oil content. According to DAS-ELISA test results PVY never present in the sunflower fields of Trakya Region. Depending on symptomatic observations and the results of TAS-ELISA tests 11 out of 244 plant samples had TSV with the rate of 4.51 %. As the results of PTA-ELISA tests and the symptomatic field observations 25 of 244 plants were found infected with Potyvirus'es with the rate of 10.25 %. Totally 36 out of 244 plant samples revealed the presence of viruses with the rate of 14.75 % in the sunflower growing areas. This is the first report of the presence of virus infections on sunflowers in Turkey. Virus infections cause reductions of 1000 seed and hectoliter seed weights of oil seeds as the oil content was found slightly high.

**Key Words :** Sunflower, *Helianthus annuus* L., ELISA, PVY, TSV, Potyvirus

# BIPOLARIS AUSTRALIENSIS ON SUNFLOWER IN RUSSIA

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## ABSTRACT

For the first time in the Russian Federation *Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama (teleomorph *Cochiobolus australiensis* (Tsuda & Ueyama) Alcorn) was found in sunflower seeds. This fungus causes human and animal diseases (allergic chronic sinusitis, dermatitis, etc.). Also *B. australiensis* is able to infect plants of different families (Fabaceae, Poaceae, etc.). On sunflower, it was previously found in India (in seeds and leaves) (Kumar and Dwivedi, 1981; Chavhan et al., 2008) and Pakistan (in seeds) (Sharfun-Nahar et al., 2005). Pathogenicity tests of our single-spore *B. australiensis* isolate under greenhouse conditions demonstrated ability of this fungus to infect healthy sunflower plants (at list in stages from seedlings to the beginning of flowering): it caused brown leaf spots. Phytotoxic property of *B. australiensis*, which was not earlier recorded for any plants, has been established. Its cultural filtrate was highly toxigenic for the 10-days-old seedlings of sunflower, having caused abnormal development of roots (up to full suppression of their growth). It has been shown that *in vitro* this dark-pigmented hyphomycete actively grew and developed on various food substrates at temperatures ranging from 4 to 40 °C, regardless of illumination (as on the light and in the darkness).

**Key Words :** Sunflower , Bipolaris australiensis, Pathogenicity, Phytotoxic property

# METABOLOMIC PROFILING OF SUNFLOWER SEEDS IN RESPONSE TO WATER STRESS DURING GERMINATION

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## ABSTRACT

Climate change is now recognised as one of the most serious challenges facing the world. In particular, climate change is a challenge for farming, and water limitation is a major abiotic stress which affects seed germination, stand establishment and *in fine* crop yields. Therefore, agricultural adaptation is necessary for the future and there is a need to better understand the molecular and cellular bases of tolerance to water stress during seed germination. In this context, tolerance to water stress during sunflower (*Helianthus annuus* L.) seed germination was studied. Seeds from one tolerant and one sensitive sunflower hybrid were selected with regards to their ability to germinate under water limitation, using a polyethylene glycol (PEG) solution (- 0.6 MPa, 20°C). A non-targeted metabolomic study was then carried out using seeds imbibed for 15 h at 20°C on water and on the PEG solution in order to identify seed metabolites associated with tolerance to water stress during the germination phase. We used liquid chromatography coupled to mass spectrometry (LC-MS) and proton nuclear magnetic resonance spectroscopy (1H-NMR). 1H-NMR spectra and the main compounds of MS spectra were annotated. Thus, 47 major compounds were selected and univariate and multivariate statistical analyses were carried out on these compounds. Statistical analyses were also performed on the entire MS profiles. Our analyses demonstrate that the metabolic profiles differ more between the two hybrids than between the two treatments. The effect of PEG imbibition was also investigated for each hybrid. We observe more response markers for the tolerant hybrid than for the sensitive one, suggesting that the metabolism of seeds from the tolerant hybrid is more affected by water stress.

**Key Words :** Sunflower, water stress, metabolomics, LC-MS, NMR