Archives of Phytopathology and Plant Protection

ISSN: 0323-5408 (Print) 1477-2906 (Online) Journal homepage: http://www.tandfonline.com/loi/gapp20

Reaction and survival of four types of sunflowers against Sclerotinia sclerotiorum under controlled conditions

Rouya Ebrahimi, Siamak Rahmanpour, Youbert Ghoosta & Mahdi Ghaffari

To cite this article: Rouya Ebrahimi, Siamak Rahmanpour, Youbert Ghoosta & Mahdi Ghaffari (2014) Reaction and survival of four types of sunflowers against Sclerotinia sclerotiorum under controlled conditions, Archives of Phytopathology and Plant Protection, 47:16, 2033-2042, DOI: 10.1080/03235408.2013.868694

To link to this article: http://dx.doi.org/10.1080/03235408.2013.868694

Published online: 20 Jan 2014.

Archives of Phytopathology and Plant Protection, 2014
Vol. 47, No. 16, 2033–2042, http://dx.doi.org/10.1080/03235408.2013.868694

Reaction and survival of four types of sunflowers against *Sclerotinia sclerotiorum* under controlled conditions

Rouya Ebrahimi[a]*, Siamak Rahmanpour[b], Youbert Ghoosta[c] and Mahdi Ghaffari[d]

[a]*Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran;* [b]*Seed and Plant Improvement Institute (S.P.I.I.), Karaj, Iran;* [c]*Department of Plant Protection, College of Agriculture, Urmia University, Urmia, Iran;* [d]*Agricultural and Natural Resources Research Station (AREO), Khoy, Iran*

(*Received 11 November 2013; accepted 16 November 2013*)

Sclerotinia wilt of sunflower caused by *Sclerotinia sclerotiorum* (Lib.) De Bary is the major disease of sunflower (*Helianthus annuus* L.) in Iran. North-western areas of Iran demonstrate the most yield losses through the disease. Development of cultivars with adequate genetic resistance is necessary to avoid these losses. Evaluation of sunflower genotypes varies based on researchers and environmental conditions. Meanwhile, greenhouse tests of the sunflower genotypes are more reliable because of controlled conditions during the assessment activities. To study the reaction of the host plants under controlled conditions, three stem inoculation techniques, including mycelium plug (MP), oxalic acid solution (OAS) and wheat seeds infested (IWS) with *Sclerotinia* mycelium, were employed. Wounded and non-wounded treatments were used in the experiment to find their effect on the disease progress. Four genotypes, including Ghalami (local variety in market), Confeta, Alstar and Master, were inoculated in this study. The factor was the lesion length to evaluate the effectiveness of different inoculation procedures. The lesion length was measured after 3, 7, 10 and 14 days post inoculation. The analysis of variance demonstrated significant differences between IWS and two other methods (MP and OAS), where the IWS produced the longest lesion lengths. In contrast, the non-significant differences between MP and OAS methods might help researchers to employ the pathogenicity factor (oxalic acid) as an alternative inoculum for their studies. Master variety was the most tolerant genotype among the treatments and its viability was 100% even at 14 days after inoculation and incubation. Interestingly, there was no mortality in all cultivars before 7-day incubations, regardless of resistant or susceptible reactions.

Keywords: Sunflower stem rot; inoculation methods; resistance; host plant viability

Introduction

Sunflower plants are of many types including those of ornamental, oil and nuts variety (Kholghi et al. 2011). This species *Helianthus annuus* L., is one of the most important economic crops in the vegetable oil industry and the fourth in the world oilseed crops (Cerboncini et al. 2002). The majority of sunflowers, as open pollinate or hybrid varieties, are categorised in the oil production group worldwide. Sunflower is widely planted in most areas of Iran, especially in the north-west parts (Kholghi et al. 2011). *Sclerotinia sclerotiorum* (Lib.) De Bary has very wide range of hosts including most of 480 plant species, and the most important agricultural host plants are beans, potato, lettuce,

*Corresponding author. Email: ebrahimirouya@gmail.com

sunflower, rape, safflower, soybean, peanuts, carrot, linseed, eggplant, cabbage, cauliflower, tomato, celery, chickpea, peas, lentil, buck wheat, capsicum, opium poppy and other vegetables (Saharan & Mehta 2008). The pathogen is an important biotic problem in world's sunflower production (Feng et al. 2005). It can attack different parts of the plant including roots, basal and mid- stem, buds, leaves and inflorescences (Godoy et al. 2005). The disease occurs as wilt or stem rot and head rot of sunflower. Wilt and basal stem rot results from root infections due to mycelium produced by the germination of the myceliogenic sclerotia. Infections appearing on the shoot parts of the host are the results of airborne ascospore (carpogenic sclerotia) distribution of the fungus. *Sclerotinia* wilt of sunflower may happen in the seedling stage but mostly wilt symptoms develop during the flowering and seed filling stages. Therefore, incidence of *Sclerotinia* wilt can severely lower the function and quality of sunflower seeds (Huang & Hoes 1980; Huang & Kozub 1991). The symptoms showing stem rot start with a small brown lesion at the base of the stem or in any part of the stem coated with white mycelium. They look stringy on severely infected plants. The pathogen may form sclerotia in the decayed parts of the stem. Cool temperatures increase the disease severity and incidence. In better word, the optimum temperature for mycelial growth is 15–25 °C, depending on the isolate, and it grows optimally on pH levels between 4 and 5.5 (Saharan & Mehta 2008). Dorrell and Huang (1978) showed wilting can be induced by a toxic metabolite which plays an important role in the development of disease symptoms. An effective and reproducible sunflower seedling resistance method through the pathogens toxic metabolites has been developed by Dorrell and Huang (1978). This toxic metabolite seems to be oxalic acid. Sunflower leaves of wilted plants show 10 times more oxalic acid than leaves of healthy plants (Noyes & Hancock 1981). Oxalic acid production is involved during plant infection as the primary determinant of pathogenicity in fungus (Thompson et al. 1995; Cessna et al. 2000; Bardin & Huang 2001). This acid has a toxic effect on host tissue and causes loss of solidarity in plant tissue by degrading calcium in the middle blade. In addition, by decreasing extracellular pH, production of cell-wall degrading enzymes is activated. Thus, the release of lytic enzymes and oxalic acid of pathogen mycelial growth is necessary frontier action between fungi and their hosts (Guo & Stoz 2007; Zou et al. 2007).

Chemical control is difficult, expensive and harmful for the environment. Thus, it is required to develop cultivars with adequate genetic resistance for the loss of performance.

Similar to canola (Bradley et al. 2006) sunflower breeding programmes need efficient, reliable and inexpensive screening method to evaluate large scales of host germplasm and cultivars for *Sclerotinia* stem rot resistance to accelerate the development of resistant sunflower cultivars. Several inoculation techniques were used for evaluating the resistance of different crops to *S. sclerotiorum*. They include mycelium plug (MP) (Vear et al. 2007; Jurke & Fernando 2008; Saharan & Mehta 2008), oxalic acid solution (OAS), which is a fairly well-known pathogenicity factor for the pathogen (Cessna et al. 2000; Rahmanpour et al. 2010; Tahmasebi Enferadi et al. 2011), and wheat seeds infested with the pathogen mycelium (Jurke & Fernando 2008; Saharan & Mehta 2008; Jones & Stewart 2011). Use of *S. sclerotiorum* mycelium or the pathogenicity factor (oxalic acid) has formed the basis of several inoculation techniques for evaluating the reaction of *Brassica* genotypes in a controlled environment. Researchers have used oxalic acid extensively for screening oilseed rape genotypes in the greenhouse (Rahmanpour et al. 2011). Bolton et al. (2006) reviewed the role of oxalic acid secreted by the fungus during pathogenesis. The relative importance of oxalic acid in

pathogenesis has been reassessed by producing mutants of *S. sclerotiorum* specifically lacking the ability to synthesise oxalic acid. These OA mutants were non-pathogenic in bioassays with *Phaseolus vulgaris* (Godoy et al. 1990). In another method, resistance of sunflower (*H. annuus*) leaf cells was evaluated to lysis in various concentrations of oxalic acid (Noyes & Hancock 1981). Tu (1989) identified tolerant and susceptible cultivars of white bean (*P. vulgaris*) based on their differences in the rate of diffusion of leaf tissue oxalic acid. These studies revealed that oxalic acid is likely a useful tool for screening cultivars and genotypes for their reaction to *S. sclerotiorum*. The objectives of this study were to find resistance reactions in two types of sunflower genotypes (industrial and confectionary) employing effective inoculation methods and to compare three inoculation techniques of sunflower stems with *S. sclerotiorum*.

Materials and methods

The sunflower varieties of the group confectionary type, including Ghalami (local variety in market), and Confeta, and the oilseed group, including Allstar and Master, were used for the experiment. Seeds of oilseed and confectionary sunflower varieties were provided by the Agricultural and Natural Resources Research Station located in Khoy, West Azarbayjan. The *Sclerotinia* isolate used in this study was collected as a single sclerot in 2010 from infected sunflowers sown in the station. The seeds of the varieties were sown in plastic pots with 15 cm internal diameter and containing a mix of soil and leaf composts (1:1). The seedlings were grown in greenhouse for 15–20 days to reach the V6–V8 growth stages in which the plants have second/third pairs of leaves and the diameter of stems are close to 6–8 mm. To inoculate the plants, three inoculation methods consisting of MP, oxalic acid treatment and wheat seeds infested with *Sclerotinia* mycelium were employed. The treatments were done on wounded and non-wounded sunflower stems with inoculums to find out the effect of wounding on disease progress.

Plug mycelium procedure

Wounded and non-wounded stems were inoculated with 5-mm diameter agar plugs containing the hyphae of the fungus *S. sclerotiorum* which had been transferred from the margins of three-day-old growing cultures. The mycelium-containing surface of the plugs was laid on the inoculation site which was the first one-third area from the crown. Mycelial plugs used by this were surrounded with small hydrophilic cotton wool balls immersed in sterile distilled water to provide high humidity. Finally, the explants were fixed with parafilm to prevent drying of the inoculum. (Vear et al. 2007; Jurke & Fernando 2008; Saharan & Mehta 2008).

Oxalic acid treatments

One ml of 10 mM OAS with pH 4.0 was poured onto a small hydrophilic cotton wool and was placed on the stem which was wounded/non-wounded at the inoculation site, which was the first one-third area from the crown. The inoculation site was then covered with parafilm to prevent drying and keep moisture. The level of pH for the acid solution was adjusted using 1 M sodium hydroxide (Rahmanpour et al. 2010; Tahmasebi Enferadi et al. 2011).

Wheat seeds infested with Sclerotinia mycelium procedure

The seeds were soaked in distilled water for two days and then autoclaved at 121 °C and 20 min for three consecutive days in 500-ml flasks. The sterile wheat seeds were inoculated with mycelial plugs (5-mm diameter) removed from the margin of the three-day-old *S. sclerotiorum* culture. Each flask had 100 g of wheat seeds and 100 ml of distilled water. The fungus and seeds complexes were incubated at 25 °C for 14–21 days until the mycelium ramified the medium without production of sclerotia. Two wheat seeds infested with the mycelium of the fungus were put on the inoculation site and covered with a piece of wet and sterile hydrophilic cotton wool. Finally, the inoculation site was sealed with parafilm to prevent drying of the inoculums (Jurke & Fernando 2008; Saharan & Mehta 2008; Jones & Stewart 2011).

In all experiments, the non-infested PDA plugs and wheat seeds were used as control treatments, in mycelial plugs and wheat seeds methods, respectively. And for oxalic acid technique, the distilled water was employed as well. Wounds on the stems were made using a disinfect surgery blade.

Three days after inoculation, parafilm of the stems were removed. The lesion length on the stem from inoculation site was measured after 3, 7, 10 and 14 days of inoculation. The experimental design was completely random with three replications. Analysis of variance was done by employing MSTATC after converting the data to logarithms.

Results

To inoculate sunflower genotypes, three experimental methods, including MP, OAS and Infested wheat seeds (IWS) were used. Symptoms resulting from the inoculation methods were observed on the stems as elongated spots, with brown margins and white centers. White-coloured mycelium of the fungus was observed on wilted stems inoculated by MP and IWS. Majority of the inoculated plants of the varieties showed wilt symptoms and died, except for the Master variety in which leaves on the wilt showing stems did not fall.

Evaluation of inoculation methods

Analysis of variance showed significant differences between the MP, OAS and IWS inoculation methods. Comparison of data showed that the maximum length of the lesion has been produced by the IWS method, The MP and OAS inoculation methods are located in same group (Table 1). All three inoculation methods showed wilt in Ghalami, Confeta and Alstar cultivars (Figure 1).

Table 1. Comparison of three inoculation methods with *Sclerotinia sclerotiorum* on sunflower cultivars under greenhouse conditions.

Inoculation method	Mean lesion length
MP	9.708 B*
OAS	8.458 B
IWS	15.694 A
Control	0 C

*Numbers with non-shared letters have significant differences.

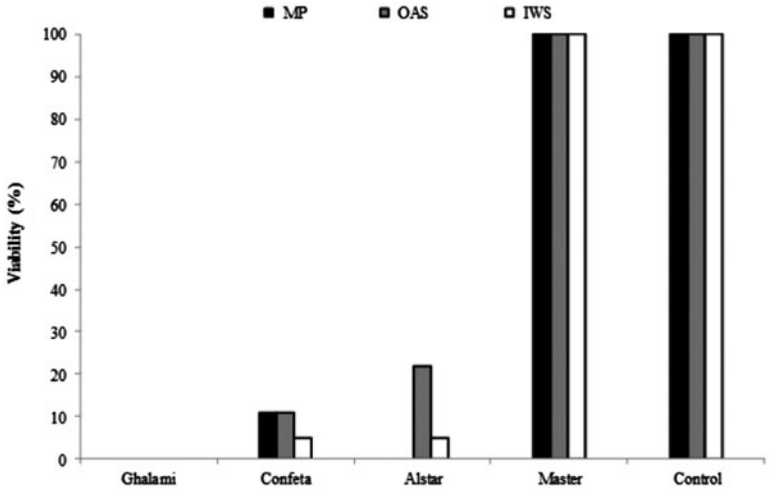

Figure 1. Viability (%) of sunflower cultivars (Ghalami, Confeta, Alstar and Master) inoculated with different inoculation methods (MP, OAS and IWS) by *S. sclerotiorum*, fourteen days incubation.

Despite wounding and non-wounding treatments for the all methods, there were no significant differences between them.

Reaction and survival of sunflower cultivars

There were significant differences between the cultivars used, Ghalami, Confets, Alstar and Master. Interestingly, each variety showed a different reaction to the various infection methods (Table 2). Maximum lesion length was observed on Alstar and Ghalami cultivars. All four varieties were infected by the three inoculation methods; meanwhile, the maximum lesion length belonged to IWS technique on the treatments (Table 3). Master was not infected with the MP technique, whereas the OAS and IWS methods produced lesions on the stems. None of the infection methods used produced wilt on the Master cultivar. In the Ghalami cultivar, 10 days after inoculation with three inoculation methods, all the plants died. Mortality was observed in plants inoculated with MP and IWS in seven days, and plants inoculated with OAS in ten days after inoculation (Figure 2). In the Confeta cultivar, 95% mortality was observed in plants inoculated with IWS method and 56% in plants inoculated with OAS 10 days after inoculation.

Table 2. Comparison of sunflower cultivars reacting to *S. sclerotiorum* under greenhouse conditions.

Cultivar	Mean length lesion
Ghalami	10.236 A*
Confeta	7.25 B
Alstar	14.139 A
Master	2.236 C

*Numbers with non-shared letters show significantly differences.

Table 3. Reactional characteristics of sunflower cultivars against *S. sclerotiorum* under different inoculation methods

Cultivar	Inoculation method			
	MP	OAS	IWS	Control
Ghalami	14.222 BC	11.444 C	15.278 A	0 D*
Confeta	8.056 B	7 BC	13.944 A	0 D
Alstar	16.556 BC	14.5 BC	25.5 A	0 D
Master	0 D	0.889 A	8.056 A	0 D

*Numbers with non-shared letters have significant differences.

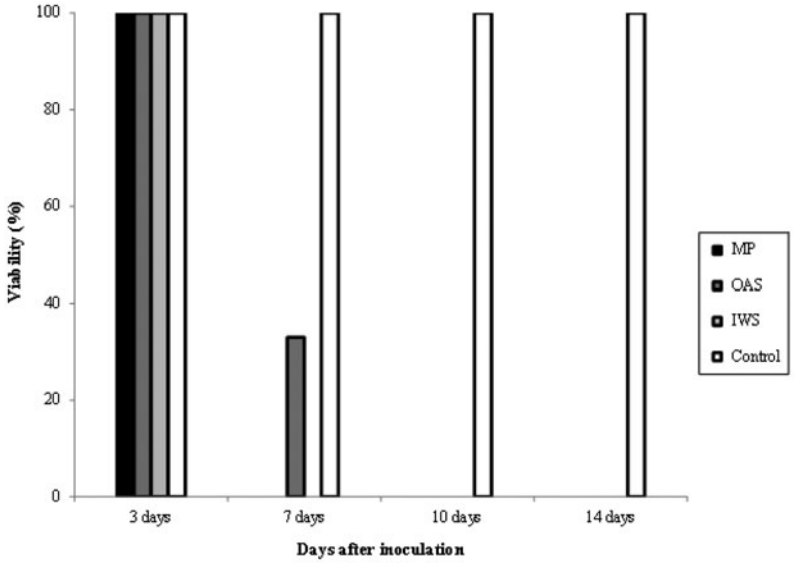

Figure 2. Viability (%) of Ghalami cultivar inoculated with three inoculation methods (MP, IWS and OAS) at during the time after inoculation.

About 89% mortality was observed in plants inoculated with MP seven days after inoculation (Figure 3). In the Alstar cultivar, 95% mortality was observed in plants inoculated with IWS method and 78% in plants inoculated with OAS 10 days after inoculation. Mortality was observed in all of the plants inoculated with MP seven days after inoculation (Figure 4). In the Master cultivar, the use of the three inoculation methods did not cause death. All plants inoculated were healthy until the last day of observation and recording of data (Figure 5). It should be noted that the lesion length created by using OAS and IWS on the stem master varieties on the third day after inoculation remained stable and did not develop until 14 days after inoculation. As a result, it can be said that the Master varieties in between these cultivars are resistant to the *S. sclerotiorum* wilt disease and the Ghalami cultivar is very susceptible to the disease. Average lesion length on the four sunflower cultivars inoculated by different methods show that the OAS and IWS cause infection on all of the inoculated plants. However, the symptoms produced by these methods differ from other cultivars on the master. The

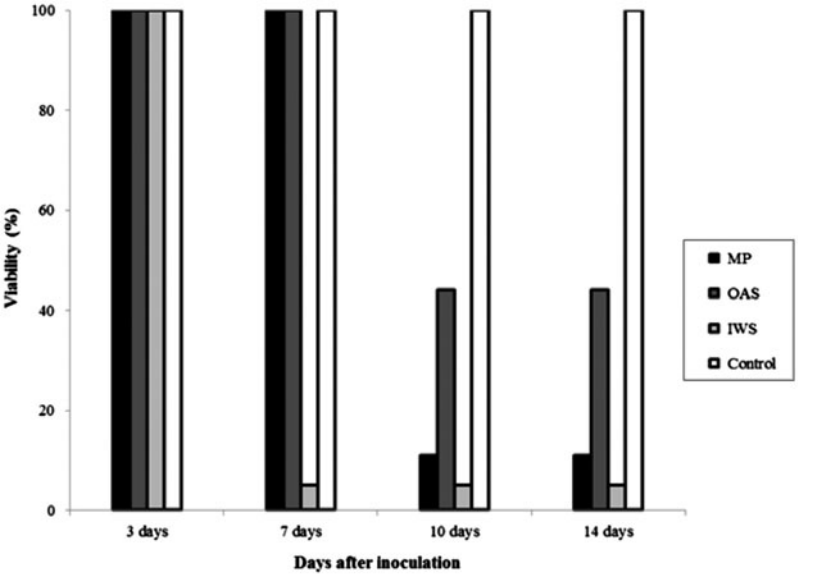

Figure 3. Viability (%) of Confeta cultivar inoculated with three inoculation methods (MP, IWS and OAS) at during the time after inoculation.

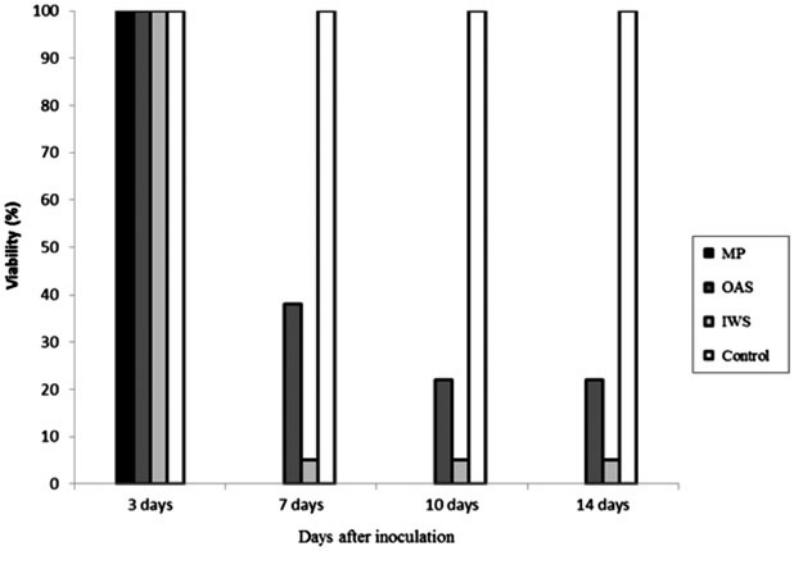

Figure 4. Viability (%) of Alstar cultivar inoculated with three inoculation methods (MP, IWS and OAS) at during the time after inoculation.

symptoms observed were the necrotic spots in the inoculated plant and progress in the amount of spots three days after inoculation to end a 14-day evaluation period. These spots had not improved and were not observed death of plants (Figure 5).

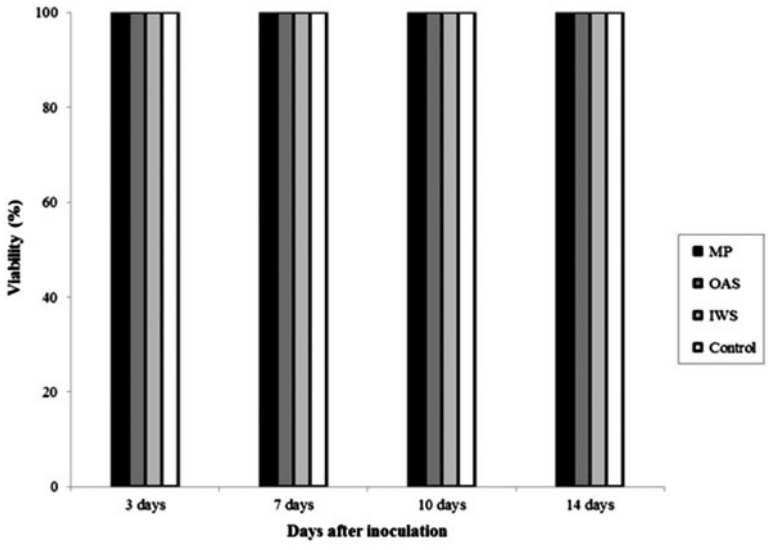

Figure 5. Viability (%) of Master cultivar inoculated with three inoculation methods (MP, IWS and OAS) at during the time after inoculation.

Discussion

Sclerotinia stem rot is a difficult disease to research in the field. The approach of consistently high levels of infection is restricted by sensitivity of the pathogen to environmental conditions (in a disease nursery). Since measurements of physiological resistance in the field can be confounded by escape mechanisms, the use of a growth room technique for *Sclerotinia* stem rot screening is favourable for achieving accuracy (Jurke & Fernando 2008).

Although resistance is assessed in the field, the results might be affected by sources of inoculum and weather conditions (Bradley et al. 2006; Li et al. 2007). In field evaluations, variable reactions to inoculation with *S. sclerotiorum* occur in this area. However, no clear reason has been reflected for this variability. Several methods have been used to identify resistance to *S. sclerotiorum* in canola. Inoculations such as detached leaf, petiole, intact leaf, stem and recently cotyledon inoculations have been used by researchers (Rahmanpour et al. 2011).

In our investigation, three inoculation techniques were employed on four varieties of sunflower to show the appearance and occurrence of the disease under controlled greenhouse conditions. There were significant differences between those methods, including IWS, MP and OAS. Data analysis showed that IWS method is a more reliable and effective method than the others under greenhouse conditions. Interestingly, wounding had no effect on the development of infection among the different inoculation techniques. This finding is supported by comparative studies performed by Auclair et al. (2004), where they applied barley kernel inoculation technique to assist soybean breeders for pre-screening physiological resistance against *Sclerotinia* stem rot. In the evaluation of greenhouse, mortality begins on the seventh day after inoculation in all methods. This case is same for the resistant and susceptible cultivars.

As the MP and OAS methods were categorised in one group, and therefore the occurrence of the conditions in which the use of fungal mycelium is prohibited, the

OAS can be used in greenhouse evaluations. These results are in agreement with Rahmanpour et al. (2011) who tested oilseed rape varieties to the same pathogen under greenhouse conditions. Oxalic acid tests have been done on detached leaves with success in soybeans. It was most efficient for growth room methods. (Kolkman & Kelly 2000). There were significant differences between the reaction of cultivars and inoculation methods observed in our studies. The variety Master demonstrated the least visible symptoms of infection during all measurement dates. To the best of our knowledge, none of the commercial sunflower hybrids possess tolerance against the disease. Thus, an extensive breeding programme is suggested to be started to transfer the tolerance from the Master variety to the newly emerged oilseed sunflower hybrids and also the confectionary materials (Ebrahimi et al. 2013).

In conclusion, among the techniques employed, inoculation method based on IWS with the fungal mycelium performed significantly for most severe infections during the evaluation of sunflower genotypes. This method demonstrates the highest infections (lesion length) of inoculation sites on sunflower stem. All four cultivars (Ghalami, Confeta, Alstar and Master) were infected by inoculation methods (MP, OAS and IWS). The maximum lesion length belonged to the IWS technique on the treatments. Master was not infected with the MP technique whereas the OAS and IWS methods produced lesions on the stems. None of the used infection methods produced wilt on the Master cultivar.

The existence of resistance to the diseases in tested varieties could be a useful opportunity for sunflower breeding programmes in controlled conditions.

References

Auclair J, Boland GJ, Cober E, Graef GL, Steadman JR, Zilka J, Rajcan I. 2004. Development of a new field inoculation technique to assess partial resistance in soybean to *Sclerotinia sclerotiorum*. Can J Plant Sci. 84:57–64.

Bardin SD, Huang HC. 2001. Research on biology and control of *Sclerotinia* diseases in Canada. Can J Plant Pathol. 23:88–98.

Bolton MD, Thomma BPHJ, Nelson BD. 2006. *Sclerotinia sclerotiorum* (Lib.) De Bary: biology and molecular traits of a cosmopolitan pathogen. Mol Plant Pathol. 7:1–16.

Bradley CA, Henson RA, Porter PM, LeGare DG, del Río LE, Khot SD. 2006. Response of canola cultivars to *Sclerotinia sclerotiorum* in controlled and field environments. Plant Dis. 90:215–219.

Cerboncini C, Beine G, Binsfeld PC, Dresen B, Peisker H, Zerwas A, Schnabl H. 2002. Sources of resistance to *Sclerotinia sclerotiorum* (Lib.) De Bary in a natural *Helianthus* gene pool. Helia. 25:167–176.

Cessna SG, Sears VE, Dickman MB, Low PS. 2000. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. plant cell. 12:2191–2199.

Chen Y, Wang D. 2005. Two convenient methods to evaluate soybean for resistance to *Sclerotinia sclerotiorum*. Plant Dis. 89:1268–1272.

Dorrell DG, Huang RC. 1978. Influence of *Sclerotinia* wilt on seed yield and quality of sunflower wilted at different stages of development. Crop Sci. 18:274–276.

Ebrahimi R, Rahmanpour S, Ghosta Y, Rezaee S, Soltani Najafabadi M. 2013. Reaction of sunflower varieties to *Sclerotinia sclerotiorum* under various inoculation methods, time of field evaluation and phylogenic stages of host. Arch Phytopathol Plant Prot. 46:825–840.

Feng J, Seiler GJ, Gulya TJ, Jan, CC 2005. Development of *Sclerotinia* stem rot resistant germplasm utilizing hexaploid Helianthus species combating *Sclerotinia sclerotiorum* (Lib.) De Bary. Department of Plant Science, University of Manitoba, Winnipeg, Manitoba Canada R3T 2N2.

Godoy G, Steadman JR, Dickman MB, Dam R. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. Physiol Mol Plant Pathol. 37:179–191.

Godoy M, Castaño F, Ré J, Rodríguez R. 2005. *Sclerotinia* resistance in sunflower: I. Genotypic variations of hybrids in three environments of Argentina. Euphytica. 145:147–154.

Guo X, Stoz H. 2007. A role for induced defense pathways in resistance to *Sclerotinia Sclerotiorum*. 2007 National Sclerotinia Initiative Annual Meeting. January 17–19; Minneapolis, MN.

Huang HC, Hoes JA. 1980. Importance of plant spacing and sclerotial position to development of *Sclerotinia* wilt of sunflower. Plant Dis. 64:81–84.

Huang HC, Kozub GC. 1991. Mono cropping to sunflower and decline of *Sclerotinia* wilt. Bot Bull Academia Sinica. 32:163–170.

Jones EE, Stewart A. 2011. *Coniothyrium minitans* survival in soil and ability to infect sclerotia of *Sclerotinia sclerotiorum*. NZ Plant Prot. 64:168–174.

Jurke CJ, Fernando WGD. 2008. Comparison of growth room screening techniques for the determination of physiological resistance to *sclerotinia* stem rot in *Brassica napus*. Arch Phytopathol Plant Prot. 41:157–174.

Kholghi M, Bernousi I, Darvishzadeh R, Pirzad A, HatamiMaleki H. 2011. Collection, evaluation and classification of Iranian confectionary sunflower (*Helianthus annuus* L.) populations using multivariate statistical techniques. Afr J Biotechnol. 10:5444–5451.

Kolkman JM, Kelly JD. 2000. An indirect test using oxalate to determine physiological resistance to white mold in common bean. Crop Sci. 40:281–285.

Li CX, Li H, Siddique AB, Sivasithamparam K, Salisbury PA, Banga SS, Banga S, Chattopadhyay C, Kumar A, Singh R, et al. 2007. The importance of the type and time of inoculation and assessment in the determination of resistance in *Brassia napus* and *B. juncea* to *Sclerotinia sclerotiorum*. Aust J Agric Res. 58:1198–1203.

Noyes RD, Hancock JG. 1981. Role of oxalic acid in the *Sclerotinia* wilt of sunflower. Phys Plant Pathol. 18:123–132.

Rahmanpour S, Backhouse D, Nonhebel HM. 2010. Reaction of glucosinolate-myrosinase defence system in *Brassica* plants to pathogenicity factor of *Sclerotinia sclerotiorum*. Eur J Plant Pathol. 128:429–433.

Rahmanpour S, Backhouse D, Nonhebel HM. 2011. Reaction of *Brassica* species to *Sclerotinia sclerotiorum* applying inoculation techniques under controlled conditions. Crop Breed J. 1:143–149.

Saharan GS, Mehta N. 2008. *Sclerotinia* diseases of crop plants: biology, ecology and disease management. Haryana: Springer Science Business Media BVCCS Haryana Agricultural University Hisar.

Tahmasebi Enferadi S, Rabiei Z, Vannozzi GP, Abbas Akbari Gh. 2011. Shikimate dehydrogenase expression and activity in sunflower genotypes susceptible and resistant to *Sclerotinia sclerotiorum* (Lib.) De Bary. J Agric Sci Tech. 13:943–952.

Thompson C, Dunwell JM, Johnstone CE, Lay V, Ray J, Schmitt M, Watson H, Nisbet G. 1995. Degradation of oxalic acid by transgenic oilseed rape plants expressing oxalate oxidase. Euphytica. 85:169–172.

Tu JC. 1989. Modes of primary infection caused by *Sclerotinia sclerotiorum* in navy bean. Microbios. 57:85–91.

Vear F, Serre F, Roche S, Walser P, Tourvieille de Labrouhe D. 2007. Improvements of *Sclerotinia sclerotiorum* head rot resistance in Sunflower by recurrent selection of a restorer population. Helia. 30:1–12.

Zou Q-J, Liu S-Y, Dong X-Y, Bi Y-H, Cao Y-C, Xu Q, Zhao Y-D, Chen H. 2007. In vivo measurements of changes in pH triggered by oxalic acid in leaf tissue of transgenic oilseed rape. Phytochem Anal. 18:341–346.