RECENT ADVANCES IN MANAGEMENT AND CONTROL OF *Fusarium* YELLOWS IN *Gladiolus* SPECIES

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ABSTRACT

Fusarium spp. causes yellowing, corm rot, browning of foliage and wilting in gladiolus. It reduces the quality, yield and market value of gladiolus. This disease is caused by the *Fusarium* species; namely *Fusarium oxysporum* f. sp. *gladioli*, *F. solani*, *F. moniliforme* and *F. roseum* in gladiolus. *F. oxysporum* f. sp. *gladioli* (Massay) Snyder and Hansen is the most common and worldwide in distribution. The fungus can survive in soil indefinitely as mycelium, clamydospores, microconidia and macroconidia. Infected corms show tissue discoloration. The corms become softened, wrinkled and mummified in storage. Despite many attempts to control this disease, the problem is still important worldwide. The management practices generally employed for its control include resistant cultivars, chemical applications, cultural practices and biotechnological approaches. However, incorporation of integrated management provides a better opportunity to manage this disease. In this review, the reports on the major progress made in management of *Fusarium* yellows in gladiolus species have been discussed.

Key words: corm rot, *Fusarium* wilt, gladiolus, management practices, pathogenicity, symptoms

INTRODUCTION

Gladiolus (*Gladiolus* spp.) belongs to the family *Iridaceae* and subfamily Ixioideae (Ranjan et al., 2010). The name gladiolus is derived from the Latin word "*gladius"* meaning sword from the shape of its leaves. The genus *Gladiolus* comprising 255 species (Goldblatt and Manning, 1998) is a very popular bulbous commercial flower. It is known for its beautiful spikes as well as long vase life (Bose et al., 2003). It is native to South Africa and has been cultivated globally. The major gladiolus producing countries are the United States, Holland, France, Poland, Italy, Bulgaria, Brazil, Australia, Israel and India. It occupies a prime position among commercial cut flowers which are in high demand in both the domestic and international market. A total of 19,900 stems of the gladiolus were imported to the European market (excluding Netherlands) at the rate of 0.52 USD per stem during 2006. Japan produced 82,760 stems of cut gladiolus domestically at the price of 0.45 USD per stem and it imported 28,800 stems from the Netherlands and Taiwan at the price of 0.27 USD per stem. Singapore imported gladiolus stem from China and Malaysia at the rate of 0.44 USD and 0.61 USD, respectively (Anonymous, 2006; Ahmad et al., 2008). The magnificent long-lasting spike of gladiolus come in a variety of colours and forms which makes it more attractive for use in herbaceous borders, bedding, rockeries, pots, as well as cut flowers (Parthasarathy and Nagaraju, 1999).

Gladiolus is susceptible to a number of diseases incited by fungal, bacterial and viral pathogens such as *Fusarium* wilt, core or spongy rot, dry or neck rot, *Curvularia* blight, bacterial scab, grey mould, storage rot etc. Pathological problems, particularly diseases caused by fungal pathogens, take a heavy toll in terms of plant stand, quality and yield (Protsenko, 1958; Vlasova and Shitan, 1974; Chandel and Bhardwaj, 2000). To date four species of *Fusarium* namely, *F. oxysporum* f. sp. *gladioli* (Massey) Snyder and Hansen, *F. solani*, *F. moniliforme* and *F. roseum* have been reported to cause wilt or yellows in gladiolus. *Fusarium oxysporum* f. sp. *gladioli* has the widest world distribution (Buxton and Robertson, 1953) and it can survive in infected corms and soil as mycelium, clamydospores, microconidia and macroconidia. *Fusarium* wilt, also known as yellows, was recorded on gladiolus as early as 1909 from California (Pryal, 1909). Later on, the disease was reported from other gladiolus growing areas of the USA (Massey, 1926). In India, gladiolus wilt caused by *F. oxysporum* f. sp. *gladioli* was first recorded by Singh (1969) from Uttar Pradesh.

Fusarium yellows is considered a serious and highly devastating disease which causes 60-70% plant mortality (Vlasova and Shitan, 1974). According to Bruhn (1955) and Protsenko (1958) about 30% annual loss was estimated in Germany and 60-80% annual loss in

Russia. In Himachal Pradesh, disease incidence ranged between 7.12-64.23%. The disease incidence is comparatively more in sub-mountainous regions than in temperate ones (Tomar, 1997). The disease has also been reported in Iraq, Taiwan, Czechoslovakia, Pakistan and other countries (Tarabeih et al., 1981; Hsieh, 1985; Vaclavik et al., 1986; Mirza and Shaker, 1991; Chen et al., 1994).

Attempts have been made to control the disease by steeping corms or cormels and drenching the soil with fungicide. The continuous use of fungicides proved to be hazardous; polluting the environment and leading to residual toxicity, creating resistance in pathogens and reducing soil fertility (Riaz et al., 2008; Nazir and Riazuddin, 2008). Therefore, the introduction of biological and cultural methods as an alternative to chemical methods, and integration of different methods together with biotechnological tools give a better opportunity to manage such diseases.

CAUSAL ORGANISM/PATHOGEN

The soil borne fungus *Fusarium oxysporum* f. sp. *gladioli* is a major causal organism of yellowing and corm rot in gladiolus (Buxton, 1955ab; Nelson et al., 1981; Dallavalle et al., 2002). *Fusarium oxysporum* and *F. solani* as the cause of gladiolus root rot from Shanghai (China) were reported by Chen et al. (1994). The same species of fungi were reported to be the causal organism of yellowing and corm rot in gladiolus (Tandon and Bhargava, 1963; Sarabhoy and Agarwal, 1983). The pathogen can also infect other members of the *Iridaceae* family (Infantino and Rumine, 1993). Tomar (1997) reported *F. moniliforme* in gladiolus as an additional fungus in causing wilt under the sub-temperate zone of Himachal Pradesh of India. Mishra and Mukhopadhyay (1999) detected root rot and wilt of gladiolus caused by *Poitrask circinons.* Chen et al. (2005) developed selective media for isolation of *F. oxysporum* by amending Komada medium (KM) with 0.1% benlate that could support good growth and high spore germination rate. The pathogen was capable of growing between pH 2 and 4. Modified KM at pH 4 recovered 96% conidia of the fungus from affected soils and gladiolus corms within 3 days. Most of the researchers have maintained the *Fusarium* cultures on potato dextrose agar (PDA) medium. Riaz et al. (2008) isolated *F. oxysporum* f. sp. *gladioli* from the diseased portions of infected corms by surface sterilizing the corms with 1% sodium hypochlorite solution followed by transfer to plates with malt extract agar (MEA) media. The plates were kept for incubation at 25 ^{o}C for 7 days. Whereas Sharma and Tripathi (2008) maintained the cultures of *F. oxysporum* f. sp. *gladioli* on PDA medium containing plates at 25±1 ^{o}C by periodic culture.

EPIDEMIOLOGY AND SYMPTOMATOLOGY

Fusarium wilt is a soil-borne disease that spreads through infected corms from one place to another (Chen et al., 1994). The initial infec-

tion of corms comes either from the soil or by latent corm infection from the previous year. Fungus is carried in practically all stocks of corms and cormels as latent infection and is also disseminated through infested soil, contaminated water and leaf hoppers.

Fusarium yellows of gladiolus show typical symptoms of yellows and wilt both in the field and under storage conditions. The fungus produces yellowing on the leaves which starts from the tip downwards (Massey, 1926). The yellowing ultimately leads to necrosis and to the death of plants. McCulloch (1944) discussed the disease symptoms on inflorescences, florets and corms. The affected florets have a reduced size and are distorted, while on corms small reddish-brown, water-soaked lesions appear which completely cover corms. The severely infected plants give a stunted appearance. The spikes are curved while roots turn into wiry strands and rot. The infected corms disintegrate completely after soft rot in the field. In storage the fungus produces brown lesions on corms which later turn into hard, dry, brownish-black structures called mummies. These symptoms have been observed by several gladiolus researchers (Buxton and Robertson, 1953; Singh, 1969; Tomar, 1997).

MORPHOLOGICAL CHARACTERS

The morphological characters of fungus have been studied in detail by Massey (1926). He observed sickle shaped macro-conidia which were curved a bit at the top, weakly pedicellate, and dominantly three septate. The micro-conidia were oval or globose, rarely septate, numerous and hyaline. Booth et al. (1978) gave a detailed account of *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme.* The morphological characters of *Fusarium oxysporum* f. sp. *gladioli* were also recorded by earlier workers (Buxton, 1955b; Chen et al., 1994). The fungus *Fusarium oxysporum* f. sp. *gladioli* (Massey) Synder and Hansen produces aerial mycelium which is hyaline, branched, septate, well-developed and cottony in appearance. The culture is slightly purple or pinkish white in colour on Potato Dextrose Agar (PDA). The fungus produces abundant conidia in culture, and conidia are of two types; micro- and macroconidia.

PATHOGENECITY TEST

The association of *Fusarium oxysporum* f. sp. *gladioli* with gladiolus yellows can be confirmed by a pathogenicity test. A healthy susceptible cultivar plant growing in pots should be inoculated with the respective fungus under controlled conditions. The typical symptoms of yellowing, browning and plant wilting will be observed after 35 days. The reisolation of a pathogen and further confirmation of its identity prove the pathogenic nature of a particular fungus (Tomar, 1997; Singh, 1969). Similar studies in proving the pathogenic nature of *F. oxysporum* f. sp. *gladioli* and *F. solani* under *in vitro* conditions were conducted by Chen et al. (1994). Bald et al. (1971) suggested

that advancement of hyphae by penetration between the cells of the vascular parenchyma which is common in isolates, causing rot in bulbs and corms, represents a helpful stage in the evaluation of the truly vascular habit among *Fusarium* spp.

ROLE OF EDAPHIC FACTORS/ SOIL ENVIRONMENT

The pathogen remains in the infected soil and corms for many years. It favours temperatures of 70 ^{o}F or above. Tomar et al. (1997) studied the effect of edaphic factors such as soil temperature, moisture, pH and soil type on the development of *Fusarium* yellows of gladiolus. Soil temperature of 27-33 °C, soil moisture of 60% and soil pH 6.5 was most conducive for the development and spread of yellows. Loam and sandy loam soils were rated as the most suitable soils for the development of the disease. Massey (1926) reported gradual increase in the rate of growth of fungus up to 35 °C with optimum at 27.5 °C and a rapid drop beyond 30 °C. Magie (1971) also reported temperature between 25-33 °C as optimum for the development and spread of the gladiolus yellows.

DISEASE MANAGEMENT

Fusarium yellows of gladiolus rapidly spreads through infected corms from one region to another or from one country to another. It considerably reduces corm and flower production in gladiolus. Therefore, it becomes necessary to manage the disease both in storage as well as in the field. Management of *Fusarium* diseases was mainly done through chemical soil fumigation and use of resistant cultivars. However, due to non availability of desirable resistance in cultivars and exorbitant cost of chemicals for the management of major soil-borne diseases, the introduction of biological and cultural methods is believed to be an alternative to chemical methods. Moreover, management practices using a single control measure are not successful in controlling the disease. For this reason, integrated management practices based on resistant cultivars, chemical method, cultural method, biological method and biotechnological approaches are being adopted for successful management of *Fusarium* diseases.

a) Resistant cultivars

Screening programs undertaken by several workers have reported resistance in many cultivars against *Fusarium oxysporum* f. sp. *gladioli* causing yellows in gladiolus. Cultivars like 'Albana', 'Apricot', 'Souvenir', 'Hopman's Glory', 'Sylvia', 'White Friendship' and 'White Prosperity' are reported to be resistant while 'Australian Fair' and 'Mansoor' were reported as tolerant to the disease (McCulloch, 1944; Bajaj et al., 1989). Tarabeih et al. (1981) found resistance in cultivar 'Mentee White' while four cultivars 'Wood Pecker', 'Lilac Wonder', 'True Love' and 'White Friendship' were reported moderately resistant to the disease. The use of resistant cultivars together with soil drench of carbendazim after 45 days of planting and

3 times at 10 day intervals was suggested by Kaur et al. (1989). Tomar (1997) also reported six cultivars 'WL-4', 'WL-5', 'Yellow Supreme', 'Rose Supreme', 'Mayur and Apollo', out of 40 cultivars resistant to disease under natural epiphytotic conditions. With the help of cross breeding, new gladiolus cultivars highly resistant to *Fusarium* were also developed by Löffler et al. (1997); Straathof et al. (1997) and Straathof et al. (1998). One of the gladiolus cultivars designated as 'Georgia Peach' is very resistant to attack of *Fusarium* spp. and *Curvalaria* spp. 'Georgia Peach' is also tolerant to the high temperatures experienced during Florida summers (August to October) and mid winter temperatures (Zipperer, 2002).

The disadvantage of the cross breeding method is that by crossing, all good parental characters segregate in the progeny. Therefore, new approaches for improvement of resistant cultivars are being used, namely *in vitro* cell line selection, molecular breeding or genetic transformation.

b) Chemical methods

The disease is controlled by treating corms with systemic fungicide before as well as after harvesting the corms. Wani et al. (1982) reported that the combination of bavistin (0.1%) and difoltan (0.5%) gave best control of wilt of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* than the use of the fungicides individually. Chauhan et al. (1988) suggested that a pre-sowing drenching with carbendazim or canboxin could be used to reduce losses, like wilt and root rot diseases, caused by *Fusarium*. Benlate (0.2%) was found superior to bavistin (0.2%) and Calixin (0.1%) according to Shah et al. (1983). Gladiolus corms treated with Sportack (0.0125%) for 3 h before planting gave good control of *Fusarium* yellows (Hsieh, 1985). *Fusarium* wilt has also been controlled by mercuric chloride, benomyl, germison, carbendazim, maneb, zineb as corm treatment and brassicol as soil disinfectant. Hanks et al. (1996) reported that doubled treatment with fungicides such as carbendazim, chlorothalonil and benomyl were very effective. The *Fusarium* pathogen usually remains in the vascular tissues near the core of the corms. Due to the presence of the suberin layer inside the corm, the fungicides usually cannot reach the core of the corms to kill the fungal pathogens. As a consequence, the fungicides are only partially effective in killing the pathogen. It has been reported that lowering the pH of the fungicide solution to 2.0-3.5 by addition of phosphoric acid, acetic acid, ascorbic acid, and ethephon softens the corm tissues and increases the penetration of the fungicides towards the core of the corms. The result is increased control of this disease (Ram et al., 2004). The softening of tissues allows the fungicides to reach the core of the corm to help destroy *Fusarium*. As a result, the effectiveness of fungicides increases appreciably in reducing the *Fusarium* corm rot disease. The treatment of corms before planting has been

proved to be better as compared to treatment of corms after harvesting (Chandel and Bhardwaj, 2000). Elmer (2006) evaluated efficacy of preplant treatments of gladiolus corms with combinations of acibenzolar-S-methyl (ASM) and biological or chemical fungicides for suppression of *Fusarium* corm rot. Corms treated with ASM produced 48% more marketable flower spikes than untreated corms and the value of the area under the disease progress curve (AUDPC) was reduced by 12%. However, chemical fungicides Medallion Reg 50WP (fludiozonil) and Terranguard TM 50WP (triflumizole) reduced AUDPC by 27% and 23%, respectively and none of the biological fungicides were effective. Fulsundar et al. (2009) found carbendazim treatment most effective in disease control as well as improving the plant height, spike length, corm weight and cormels per plant.

Indiscriminate use of these chemicals has often resulted in adverse environmental effects, disturbing the ecological balance of soils and making plants even more resistant to pests and pathogens. Increased public concern on environmental issues requires alternative disease management based on cultural practices and naturally occurring compounds.

c) Cultural methods

The control methods that depend primarily on certain actions of growers are known as cultural practices such as crop rotation, early lifting, sanitation, and proper curing of the corms at 29.5 °C to 30 °C for about one week. Removal of corm scales before planting (Woltz et al., 1978), adjustment of soil pH (6.5-7.0), soil nitrogen availability (80-90%), adequate aeration in storage buds, improved water drainage and incorporation of organic amendments (Sharma and Bedi, 1988; Linderman, 1989), are of great importance. Use of S-H mixture containing bagasse, rice husks, oyster, shell powder, urea, KNO_3, calcium super phosphate and mineral ash suppressed the fungus growth due to germ tube lysis and spore inhibition of germination. Population of *Fusarium* spp. decreased with wheat straw, ginseng leaves and cabbage compared to the untreated control (Soni et al., 1985). Bhardwaj et al. (2000) reported the effect of sowing date and fungicidal effect on management of *Fusarium* wilt of gladiolus. Raj and Upmanyu 2006 studied the effect of solarization of soil amended with residues of cabbage leaves and corm treatment with fungicides, for management of wilt of gladiolus. The residues of six crucifer crops (broccoli, cabbage, *Brassica chinensis*, cauliflower, radish and Indian mustard) were tested under solarised conditions against the wilt. Cabbage was considered the most effective resulting in 75-87.6% inhibition of mycelial growth compared to the non-solarised control. Riaz et al. (2009) conducted pot and field experiments to study the effect of co-cultivation and crop rotation on corm rot of gladiolus. In the field experiment, gladiolus was co-cultivated with 10 agricul-

tural/horticultural crops viz. *Allium cepa* L., *Brassica campestris* L., *Capsicum annuum* L., *Eruca sativa* Mill., *Helianthus annuus* L., *Tagetes erectus* L., *Zea mays* L., *Vinca rosea* L. and *Rosa indica* L., in a soil infested with *F. oxysporum.* All the crops except *V. rosea* and *R. indica* reduced disease incidence. The effect of *H. annuus* and *T. erectus* was significant and more pronounced than other co-cultivated crops. In general, root and shoot dry biomass, corm fresh weight, number of cormlets and number of flowers per spike decreased as compared to the uninoculated monoculture gladiolus treatment (negative control) but these parameters were enhanced compared to the *F. oxysporum* inoculated monoculture gladiolus treatment (positive control). In the pot experiments all the crops of the field experiment, except *V. rosea* and *R. indica,* were sown in rotation with gladiolus. Disease incidence was significantly suppressed in all the treatments ranging from 29% to 53%. The highest suppression of disease incidence was recorded in *T. erectus* (53%) followed by *B. campestris* (49%). Corm rot disease of gladiolus can be managed by mixed cropping of *H. annuus* and *T. erectus* or cultivation of *T. erectus* and *B. campestris* in rotation.

d) Biological methods

Biological control includes total or partial destruction of a pathogen population by other microorganisms. *Fusarium oxysporum* thrives well and causes several diseases in some soils known as conducive soils. It develops much less and causes much milder disease in other soils such as Lateritic clay soil, known as suppressive soil. The suppression of disease is due to the presence of microbiota especially *Streptomyces* spp. in Lateritic clay soil that suppress infection. Inoculation of freshly harvested cleaved corms with an isolate of *F. monliforme,* controlled corm rot (Woltz et al., 1978; Magie, 1980). Mohamed and Gomaa (2000) studied the effect of bioagents and agricultural chemicals on *Fusarium* wilt incidence and growth characters of gladiolus plants. For efficacy, two biological control agents (BCA) i.e. *Trichoderma harzianum* and *Bacillus subtilis* and two agricultural chemicals (sulfur and lime-calcium hydroxide) were compared with recommended fungicides Vitavax (captan) 75wP and Rizolex-T50 (Tolelofos-methyl + thiram), as soil or corm-soaking treatments in controlling *Fusarium* disease on gladiolus 'Peter Pears'. The effects of these agents, chemicals and fungicides on several plant growth parameters were also studied. It was observed that most of the treatments decreased the diseased corm number, as well as increased the survival of the plant growth and corm formation. Growth inhibition of pathogen by *Trichoderma harzianum*, *T. viride* and *T. virens* has been reported by Sharma and Chandel (2003). This inhibition can be attributed to antibiosis. A similar antagonistic activity of *Trichoderma* spp. was reported by Dennis and Webster (1971) and

Mukhopadhyay and Mukherjee (1996). Mishra et al. (2005) reported integrated and biological control of gladiolus corm rot and wilt caused by *Fusarium oxysporum* f. sp. *gladioli*. Isolate of *Trichoderma virens* Miller, Giddens & Foster and carboxin, individually and in combined form, significantly reduced disease incidence in both glasshouse and field conditions. Roebroeck and Mes (1992) also obtained reduction in wilt incidence with non-pathogenic *Fusarium* isolates before corms were dipped in a spore suspension of non-pathogenic *Fusarium* isolate and incubated under moist conditions at 20 °C, to achieve good biological control agent results. Sharma and Chandel (2006) reported biological control of gladiolus wilt caused by *Fusarium oxysporum* f. sp. *gladioli* using different methods. The soil placement method proved effective compared to the corm dip method. *T. harzianum* in comparison to *T. viride* performed superior against wilt pathogen and resulted in minimum disease incidence in addition to improvement in growth parameters of gladiolus. An attempt to manage the disease with AM (Arbuscular mycorrhiza) fungi (*Glomus mosseae* and *G. etunicatum*) under pot culture conditions was made by Bhardwaj et al. (2000). Plants inoculated with *Glomus mosseae* resulted in maximum disease reduction followed by *G. etunicatum.* Significantly higher root length and root dry weight was recorded in mycorrhizal inoculated plants than non-mycorrhizal plants. Anand and Gautam (2006) reported use of soil solarization, fungicide corm dip and soil amendments for *Fusarium* wilt. Corms were treated with carbendazim, carbendazim + mancozeb (0.2%) or *T. viride* formulation (0.5%) before sowing. Three fungicide drenches at 10 day intervals for a month after sowing of corms were found quite effective. Soil amendments with cabbage leaf residue together with soil solarization were found to be the most effective treatment for disease control (98.5%). Kulkarni et al. (2007) reported screening biocontrol agents and cultivars. The maximum reduction in colony size was observed in *T. harzianum* (76.08) which was significantly superior over all other bioagents tested. The second best was *T. konigii* (72.48%) followed by *T. viriens* (66.30%) and *T. viride* (61.44%) while *B. subtilis* and *Pseudomonas fluorescens* were least effective in inhibiting mycelial growth.

The plant world is composed of a rich storehouse of biochemicals to be used as pesticides which are more environmentally safe than chemical alternatives (Hashim and Devi, 2003). Plants are the reservoirs of biodegradable secondary metabolites that are reported to inhibit various phytopathogenic fungi. Riaz et al. (2008) obtained antifungal activity of plant extracts against corm rot. Antifungal activity of different concentrations (2, 4, 6 and 8% w/v) of leaf extracts of wheat (*Triticum sativum*), maize, sunflower, chillies, onion (*Allium cepa*) and marigold (*Tagetes erectus*) was studied. The conclusion was that extracts of marigold, sun-

flower and chillies were highly efficient as the employed extract concentrations which significantly reduced fungal biomass by 54-79%, 33-85%, 45-57% respectively. There are several other reports on antifungal activity of phytochemicals against pathogenic fungi (Charmaine et al., 2005; Bajwa et al., 2008). The inhibition of mycelial growth of fungus has been observed by different phytoextracts of *Azadirachta indica*, *Ocimum sanctum* and *Allium sativum* (Tomar and Chandel, 2006). Nagesh et al. (1998) reported integrated management of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *gladioli* in gladiolus using antagonistic fungi and neem cake. Both *T. harzianum* and *T. viride* controlled wilt in the presence of *M. incognita* and must be applied 6 weeks prior to plant emergence. Further greenhouse and field trials must be investigated for commercial usage of these phyochemicals against corm rot disease of gladiolus.

Essential oils are complex volatile compounds. Their constituents have been used as biological agents due to their therapeutic activity and toxicity against insects as well as plant pathogenic fungi (Delespaul et al., 2000). They are needed to reduce the use of chemical in agriculture. There had been an increased interest in the possibility of the application of essential oils to control plant pathogens. The essential oils of *Thymus vulgaris* has been reported to inhibit fungal growth. Their fungistatic activity is due to the presence of thymol at 50.06% in the tested oil (Zambonelli et al., 1996). The oil of *Chenopodium ambrosioides* can completely inhibit the mycelial growth of *Aspergillus flavus* Link and *Fusarium oxysporum* at 100 ppm (Kumar et al., 2007). The essential oils of *C. zeylanicum*, *S. aromaticum* and *T. vulgaris* has capability of totally inhibiting the mycelial growth of *Fusarium oxysporum* f. sp. *gladioli*. The compounds such as carvacrol, geraniol and trans-cinnamaldehyde provide a high antifungal activity against this fungus (Barrera-Necha et al., 2009).

e) Integrated management

Control of plant diseases is most successful when all available information regarding the crop, its pathogen, environmental conditions, control measures and their costs are taken into account for controlling the disease. Integrated management of *Fusarium* yellows of gladiolus under pot culture and polyhouse conditions has been achieved by Sharma et al. (2005). The results of an integrated approach using pots treated with neem cake, carbendazim and *Trichoderma harzianum* revealed the highest disease control. This approach enhanced corm yield and improved plant health. Mishra et al. (2005) studied the effect of integration of chemicals and biological control agents against gladiolus corm rot. An isolate of *T. viriens*, carboxin, and a combination of both were evaluated for control of gladiolus corm rot and wilt. Chandel and Tomar (2007) evaluated fungicides and biopreparations against *Fusarium* wilt of gladiolus. According to their obser-

vations corm rot treatment and soil drenching with Quintal were found effective in reducing wilt incidence to 11.6%. Achook, a biopreparation, gave significant control of wilt followed by Neemazol and Nimbicidine. Sharma and Tripathi (2008) reported integrated management for post harvest *Fusarium* rot of gladiolus corms. Gladiolus corms artificially inoculated with the pathogen *Fusarium oxysporum* f. sp. *gladioli* were treated with hot water, UV-C or essential oil of *Hyptis suaveolens* (L.) poit, alone and in combinations. The population growth of the pathogen after storage for 4 and 12 weeks in UV-C or essential oil treatments, resulted in reduction of corm rot incidence. It was observed that a hot water treatment at 55 °C for 25 min or a UV-C treatment with a dose of 3.63 KJm^{-2} were sufficient to inhibit germination of conidia.

f) Biotechnological approaches

Breeding for disease resistance through conventional techniques is a long term program which is a continuous and laborious task requiring patience and persistence. Plant cell culture can help to supplement the efficiency of specific steps in the overall breeding procedure. It is now well established that *in vitro* culture of higher plants can be exploited for genetic variation and selection of mutants. Using *in vitro* cell selection techniques, mutants have been obtained for resistance against toxins of pathogen. Selection for resistance is the most straightforward method for mutant selection. In this method, resistant cells in large populations can be selected for their ability to grow in the pressure of toxin inhibitor. Apart from cell line selection procedures, molecular breeding is being used to improve the disease resistance in elite genotypes. However, these techniques require an efficient *in vitro* regeneration method. These techniques also require identification of genes responsible for conferring disease resistance which may be eventually used for genetic transformation of gladiolus for disease resistance.

***In vitro* selection**: Plant cell culture provides a unique opportunity to manipulate morphogenesis in a controlled environment which is a powerful complementary tool for crop improvement. Since the late 1970's the process of *in vitro* selection had been applied to several cell culture systems to generate mutants with useful agronomic traits such as disease resistance. However, the promise of genetic engineering technology, and some early failures among the *in vitro* selected plants, shifted the focus of research in this area. Recent advances in molecular characterization of stress-related response and the emergence of sensitive molecular analytical tools have been emerging for use in research on *in vitro* selections. This technology is easy to use, and not encumbered by intellectual property issues and social concerns currently inhibiting the development of transgenic crops. Thus, it is an attractive complement to existing crop improvement strate-

gies. *In vitro* methods for the selection of mutants offer several important advantages over their *in vitro* counterparts (Gunn and Day, 1986). *In vitro* selection for *Fusarium* resistance in gladiolus was investigated by Remotti et al. (1997). According to them, *Fusarium* resistant cultivars were able to tolerate higher concentrations of the toxin than susceptible ones. The cell suspension was challenged stepwise with increasing concentrations of fusaric acid (0.12 mM and 0.4 mM). Nine cell lines selected on 0.12 mM fusaric acid showed variable reactions when inoculated directly with conidia of the fungus. The growth of the fungus was reduced by at least 50% compared to that on non-selected callus, and 50% of the plantlets regenerated from selected calli showed increased tolerance to the toxin. Pathania and Misra (2002) obtained resistant cultivars of gladiolus against fungus using *in vitro* selection by challenging with fusaric acid (1-1.5 mM) and culture filtrate (20%). The results at the end of the 3rd selection cycle were mutants insensitive to *Fusarium* yellows. *Ex vitro* evaluation further confirmed that fusaric acid and culture filtrate were suitable phytotoxins for selecting optimal resistance against *F. oxysporum* f. sp. *gladioli.*

Kanwar et al. (2003) reported cellular selection of gladiolus to *Fusarium oxysporum* f. sp. *gladioli*. Nasir and Riazuddin (2008) also studied *in vitro* selection of gladiolus against the pathogen. Cell suspension of four *Fusarium* susceptible gladiolus cultivars ('Friendship', 'Peter Pears', 'Victor Borge' and 'Novalux') were found highly sensitive to fusaric acid. Gradual increase in fusaric acid (FA) concentrations to the cell suspension cultures decreased cell growth considerably. Plantlets of all the selected cell lines showed significant resistance compared to the control in the presence of 0.5 mM FA. The *in vitro* selected cell lines showed significant resistance as compared to the control in the presence of 0.5 mM FA. The *in vitro* selected cell lines numbers CAMB-G01, CAMB-G04, CAMB-G06 and CAMB-G09 were not affected at all and showed an average severity index of zero compared to the control of the 'Friendship' cultivar. Plantlets of all selected cell lines exhibited significant growth when compared to the control, after application of conidia of *Fusarium oxysporum* f. sp. *gladioli.*

Genetic transformation for disease resistance: The prerequisite for successful transformation of plant species for the introduction of desirable characters is the availability of reproducible, efficient and robust *in vitro* plant regeneration procedure. Induction of callus from various parts of gladiolus was attempted by several researchers (Kim et al., 1988; Remotti, 1995; Remotti and Löffler, 1995). Many other studies were carried out on plant regeneration from callus as well as suspension cultures of gladiolus (Kamo, 1994; Kamo and Vaneck, 1997; Kasumi et al., 1998; Kumar et al., 1999; Kamo and Joung, 2007). Direct DNA transfer using callus as well as cell suspension cul-

tures through biolistics have already been reported (Kamo and Lawson, 1995; Kamo et al., 2000; Löffler et al., 2000). Transgenic gladiolus plants thus obtained, were grown in the greenhouse under federal guidelines for containment of genetically engineered plants. Ultimately though, transgenic gladiolus plants will be grown outdoors. Keeping this in mind, Kamo (2008) studied transgenic gladiolus plants expressing three different transgenes under the control of four different promoters. This was done to evaluate if transgene expression would change significantly when the greenhouse grown plants were grown outdoors. The transgene expression continued for two seasons for gladiolus plants grown in the greenhouse and outdoors. Silencing of the expressing lines was not observed during the two years of growth in the greenhouse and outdoors. Transgene expression was higher in 3 out of 12 plant lines grown outdoors as compared to 1 out of 12 plant lines grown indoors. It was concluded, that there are other factors each contributing differently to transgene expression for each transformed line. These include such factors as location of transgene in genome, which may affect endogenous genes and their expression.

Molecular approaches: A variety of *Fusarium oxysporum* f. sp. *gladioli* resistance tests, based mainly on biological assays using cultivars and species of gladiolus have already been reported (Löffler et al., 1997; Straathof et al., 1998). However, the resistance mechanisms are still poorly understood (Remotti and Löffler, 1996). Mycelium of the causal pathogen has been observed to penetrate host tissues only through discontinuity sites in the corm periderm, mainly at root formation in the basal crown, nodes, and wounds (Dallavalle and Pisi, 1993). The tissue of more resistant cultivars reacts with cell suberization, forming barriers that inhibit fungal colonization. In recent years, plant pathologists have been interested in understanding the disease resistance pathways and the genes involved in providing resistance. These pathologists have started using various molecular techniques. Molecular markers such as allozymes, restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) have been extensively used to characterize pathogen populations. Appropriate sampling and use of molecular markers allows plant pathologists to make inferences about pathogen biology and evolution which is relevant to plant disease control. RAPD marker is useful in describing the origin and the phylogeny of isolates collected from different origins/regions. It is used in genetic diversity and genetic distance related study of various pathogens.

De Haan et al. (2000) had developed multiplex PCR assay for detection of *Fusarium oxysporum* f. sp. *gladioli* race 1 from infected corms. Dallavalle et al. (2002) performed RAPD analysis of the genomes of 9 gladiolus cultivars with varying degrees of sensitivity to *Fusarium oxysporum* f. sp. *Gladioli*. Their intentions were to

determine the possibility of applying DNA screening methods to discriminate between sensitive and resistant gladiolus cultivars. The objective of their study was to differentiate between sensitive and resistant gladiolus cultivars using DNA based analysis. About 14 primers with varying *Taq* polymerases and primer concentrations were used for the RAPD analysis. Only five primers produced polymorphic bands and all the tested growth stages provided similar results. It has been shown that different tools are valuable in investigating the variability of this fungus. Molecular techniques are very useful for the detection of a pathogenic group of *Fusarium* sp. isolates facilitating a preventive approach to the disease. These tools integrate the knowledge obtained from pathogenicity tests etc. and make it possible to propose a hypothesis on the phylogenetic relations between isolates. Microsatellite marker technology is based on identifying highly conserved gene sequence of a concerned organism. Virulence factor gene related microsatellite marker is a valuable tool to get the information that the virulence gene sequences are a highly conserved region. These virulence markers are associated with the virulence/pathogenic nature of the pathogen.

CONCLUSION

Gladiolus being an important commercial flower is fetching high returns to the growers in national and international markets. The occurrence of *Fusarium* wilt in devastating form, has become a limiting factor in its production. The pathogen is soilborne in nature, hence difficult to control. Chemical control has been a routine practice in its management. But, use of chemicals is associated with many bad effects such as the development of resistance to pathogen, new races of pathogen, depleting of soil fertility, hampering beneficial microbiota and negative effects on the environment as a whole. Searching for alternatives in the disease management of *Fusarium* wilt of gladiolus would be an appropriate approach. Use of resistant cultivars, biocontrol agents, cultural practices and the most recent biotechnological tools, in one form or combined forms, can enhance the efficiency of management strategies against *Fusarium* wilt diseases. Introduction and evaluation of new technology like RAPD marker, microsatellite marker and Virulence factor gene related microsatellite marker techniques in agricultural systems will certainly influence the biotechnological way. These new technologies will be performed in the near future for assessing the intra- and interspecific identification of *Fusarium* wilt pathogens of gladiolus. In addition to increasing the understanding of the disease for improving crop productivity, these results can be explored for developing integrated strategies for disease management.

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NAJNOWSZE POSTĘPY W KONTROLI I OCHRONIE PRZED *Fusarium* YELLOWS U MIECZYKA

Sunita Chandel i Raj Deepika

S T R E S Z C Z E N I E

Fusarium spp. powoduje żółknięcie, gnicie bulw, brązowienie liści oraz więdnięcie mieczyka, co zmniejsza jego jakość, plon oraz wartość rynkową. *Fusarium oxysporum* f. sp. *gladioli*, *F. solani*, *F. moniliforme* oraz *F. roseum* należą do gatunków *Fusarium* powodujących tę chorobę u mieczyka. Najbardziej znany i rozpowszechniony na świecie jest grzyb *F. oxysporum* f. sp. *gladioli* (Massay) Snyder i Hansen. Grzyby te mogą występować w glebie nieprzerwanie jako grzybnia, chlamydospory, mikrokonidia oraz makrokonidia. U zarażonych bulw możemy zaobserwować odbarwienie tkanki. Bulwy te w czasie magazynowania stają się bardziej miękkie, pomarszczone i wyschnięte. Pomimo licznych prób zapobiegania tej chorobie, wciąż stanowi ona poważny problem na całym świecie. W walce z nimi powszechnie stosowane są następujące działania ochronne: zastosowanie odpornych odmian i środków chemicznych, zabiegi uprawowe oraz podejście biotechnologiczne. Lepsze możliwości zwalczenia tej choroby daje jednak włączenie zintegrowanej ochrony. W pracy tej przedstawione są główne postępy w działaniach mających na celu ochronę przed *Fusarium* yellows u mieczyka.

Słowa kluczowe: gnicie bulw, fuzaryjne więdnięcie, mieczyk, działania ochronne, patogenność, symptomy