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Review paper

The Use of Vegetative Compatibility Tests for Identification of Biodiversity of Phytopathogenic Fungi

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SUMMARY

Visual assessment of phenotypes, performed when two strains of one fungal species are cultivated in a mixed culture on specific media, is known as vegetative or heterokaryotic compatibility or incompatibility test, which enables identification of fungal clones and their classification based on phylogenetic groups. Hyphae of strains that have identical alleles at all *vic* loci can anastomose into a form of a visible heterokaryon. Strains that divide compatible loci and can anastomose each other belong to a subpopulation termed the vegetative compatibility group (VCG), which is genetically distinguishable from other VCGs. Each VCG is specific regarding its host plant or related host groups and can, but does not have to be virulent on other hosts.

Vegetative compatibility can be established in different ways, but complementary auxotrophic strains or strains formed by spontaneous mutation during nutrition, capable of forming a prototrophic heterokaryon are predominantly used. The *nit* mutants are considered excellent genetic markers for determination of vegetative compatibility and grouping of strains or clones of one fungus into the same or different VCGs. The ability only to determine whether strains are the same or not, but not the degree of their relatedness using VCG, is a limiting factor in analyses that could be performed. VCGs are the most efficient when they are employed to detect the presence of a specific strain in a population.

This paper provides an overview of the importance of the phenomenon of vegetative compatibility. Vegetative compatibility is one of the most important genetic traits in ascomycetes by which one subpopulation can be identified as a distinct genetic group. Furthermore, the procedures for isolation, identification and determination of *nit* mutant phenotypes, and for identification of complementary strains and VCGs are described in detail.

Keywords: Vegetative compatibility; Phytopathogenic fungi; Genetic diversity; VCGs

INTRODUCTION

The majority of fungi have been thought to be asexual and clonal, while recent studies have shown that they also recombine. Today, mutation, heterokaryosis and somatic recombination are known to be the principle mechanisms of nuclei variation in fungi imperfecti (*Fungi imperfecti*), the same as sexual reproduction of fungi of other classes (Taylor et al., 1999).

For the most part of their life, vegetative hyphae of numerous fungi are haploid and do not have sexual reproduction. During its vegetative state, a fungus is able to undergo hyphal fusion, karyogamy and mitotic recombination in a so-called parasexual cycle. Finding an appropriate partner for mitotic recombination is a crucial aspect of the parasexual cycle, similar to the sexual mating process (Pál et al., 2007). When two haploid mycelia of different genotypes are fused, a mycelium with two nuclear types is formed; in this mycelium, two haploid strains coexist and divide mitotically within the common cytoplasm. Such a set of cells that contain multiple, genetically different nuclei in their common cytoplasm is called heterokaryon. The fusion of nuclei or diploidisation can rarely occur, whereby a heterozygous diploid nucleus is formed and mitotically divided. Crossing-over occurs very seldom in mitosis. By division, diploid nuclei regain haploid properties (haploidisation). In the course of these mitotic divisions of diploid nuclei, recombination can occur between homologous chromosomes. Thus, the parasexual system is a system in which genetic recombination occurs in a different way than meiosis (Muntanola-Cvetković, 1990).

Heterokaryons are often characterised by a stronger "heterokaryotic power", hence they prevail in reproduction over homokaryotic strains. If in such a way more stable strains are obtained, especially after a fusion of heterokaryons into a single diploid nucleus known as "synkarion", it enables differentiation of more active strains in terms of biochemistry. This is important not only in studying the variability of microorganisms but also for their utilisation in industrial processes. This phenomenon of mitotic recombination is widespread among imperfect fungi (*Deuteromycetes*, *Fungi imperfecti*) that lack sexual reproduction. It is believed that a similar phenomenon exists in other phyla of fungi in the vegetative stage and actinomycetes (Tešić and Todorović, 1988). Mitotic recombination was initially detected in *Aspergillus nidulans* (Eidam) Winters, *A. niger Tiegh.* and *Penicillium chrysogenum* Thom (Muntanola-Cvetković, 1990).

The initial step in the parasexual cycle of fungi (prefusion) is controlled by genes involved in pheromone production and receptors, and in heterokaryon self-incompatibility (*hsi* genes). Heterokaryon incompatibility (*het*) or vegetative incompatibility (*vic*) genes are responsible for the fusion step, in which the interhyphal anastomoses are formed (Leslie, 1993). After fusion, cell death may be associated with suppressor (*sup*) genes, which can modify the signal. In the final step of the parasexual cycle of fungi, programmed cell death genes initiate processes leading to apoptosis (Pál et al., 2007).

VEGETATIVE COMPATIBILITY (OR INCOMPATIBILITY) AND VEGETATIVE COMPATIBLE GROUPS (VCGs)

Fungi can be considered as fluid, dynamic systems characterised by hyphal tip growth, branching, and hyphal fusion (anastomosis). Hyphal fusions are a way to increase protoplasmic flow restricted by septa, which may be important in the formation of mycelium. The viability of heterokaryons depends on the genetic constitution at heterokaryon incompatibility (*het*) loci or *vic* loci (Glass et al., 2000). Somatic (vegetative or heterokaryonic) compatibility has a long history in mycological and genetic research, and has been studied in many ascomycetes (Leslie, 1993). Simply said, vegetative compatibility means that two hyphae can anastomose and fuse in the form of a viable heterokaryon and on such spot they can exchange cytoplasmic or nuclear content. Fungal strains that form viable heterokaryons are called vegetative compatible strains and are classified into the same vegetative compatible group (VCG). On the other hand, strains not capable of forming such heterokaryons are vegetatively incompatible and classified into different VCGs (Correll et al., 1987; Jacobson and Gordon, 1988).

Alleles at all *vic* loci have to be identical for successful heterokaryosis (Vaillancourt and Hanau, 1994). Allelic reaction of vegetative compatibility has been described in various fungal genera, such as *Aspergillus*, *Ceratocystis, Cohliobolus, Colletotrichum*, *Cryphonectria*, *Cryptostroma, Diaporthe, Fusarium, Hypoxylon, Leptographium, Leucostoma, Leucocytospora, Morchella, Neurospora, Penicillium, Podospora, Sclerotinia, Septoria, Trichoderma* and *Verticillium* (Leslie, 1993). At least 10 different vegetative incompatible (*vic*) loci have been identified and 5 have been mapped in *Neurospora*, and 8 *vic* loci are known in

Aspergillus nidulans. Furthermore, there is evidence of genetic segregation of *vic* loci in *Fusarium verticillioides* (Sacc.) Nirenberg – syn. *F. moniliforme* Sheldon (telemorph: *Gibberella moniliformis* Wineland) and *F. graminearum* Schwabe (telemorph: *Gibberrela zeae* (Schw.) Petch). Since the number of *vic* loci is great (10-15 in the majority of ascomycetes), the possible number of VCGs is also high (thousands) (Leslie and Summerell, 2006). The progeny derived from parental lines with the same alleles in all *vic* genes belong to the same VCG. If the progeny belongs to two VCGs it means that parents had different alleles at one locus (2^1). Differences at two loci should give four types of progenies (2^2), while differences at three loci result in eight types of progenies (2^3), etc. (Anagnostakis, 1982). There are at least 10 known *vic* loci regulating vegetative compatibility in *F. moniliforme*. The 10 *vic* loci, each with two distinguishable alleles, can produce by sexual recombinations 2^{10} (1024) different arrangements that theoretically can give an identical number of VCGs (Elmer, 1991). The higher the number of strains and the greater diversity of geographic origin of strains, the greater the number of VCGs. Strains within the form species are not always monophyletic in origin or genetically uniform (Baayen, 2000). If two strains are clones of a related strain they will always be in the same VCG, but strains in the same VCG do not have to be clones. Thus, the number of identified VCGs in a sample is a minimum number of genetic individuals, for instance haplotypes in the sample (Leslie and Summerell, 2006).

Several studies have shown that strains of similar pathogenicity belong to the same VCG (Strausbaugh et al., 1992; Daayf et al., 1995; Dervis et al., 2009). Strains belonging to *Fusarium oxysporum* f. sp. *cepae* W.C. Snyder are genetically similar, probably originating from one pathogenic genotype or from the same clonal lineage (Swift et al., 2002). Kistler (1997) assumed that VCGs within the population *F. oxysporum* Schlecht. were separate clonal lineages. Woo et al. (1996) reported that a correlation between VCG and geographic origin was not determined in *Fusarium oxysporum* f. sp. *phaseoli* J.B. Kendr. & W.C. Snyder. In addition, Krnjaja (2005) and Krnjaja et al. (2007a, 2007b, 2008, 2012) studied genetic diversity in several *Fusarium* species and did not determine common VCGs within one species originating from various locations in Serbia.

In an asexual population, such as is thought to exist for *F. oxysporum*, there should be no sexual recombination, and strains with similar pathogenicity appearing as part of a clonal lineage should all be in the same VCG. Thus, if there were a rapid way to identify VCGs, these identifications could serve as a substitute for long-term pathogenicity tests and provide fast enough analyses for evaluation of isolate populations. These ideas have been initially confirmed in experiments conducted by Puhalla et al. (1985). The main obstacle was the identification of mutants that could be used to force heterokaryons and show the occurrence of heterokaryosis.

The strategy for VCG identification is the isolation of *nit* mutants (nitrate non-utilising mutants) as spontaneous chlorate-resistant sectors, and then their differentiation (identification) based on their growth on media that contain various nitrogen sources. Mutants can be paired on a favourable nutritive medium, usually a minimal medium (MM) with nitrate (NO_3) as the only nitrogen source and then vegetatively compatible strains can be easily identified. Based on the above stated, numerous studies on vegetative compatibility of many ascomycetes, especially for the *F. oxysporum* species, have been carried out (Leslie and Summerell, 2006).

Complex relationships among pathogenic races and VCGs (or clonal lineages) exist in *formae specialis* of *F. oxysporum*. One race can be present in more than one VCG or one VCG can encompass more races. One interpretation of these relationships is that a given pathogenic race has multiple origins or that different pathogen races may be closely related (Gordon and Martyn, 1997).

Host specificity and pathogenicity genes in different VCGs of a *forma specialis* are dissimilar generally, rendering the *forma specialis* concept artificial and of little predictive value. The practical diagnostic value of such an artificial classification system will undoubtedly eventually prove inferior to a novel, VCG-based and more natural system with greater predictive value than host specificity (Baayen et al., 2000).

Fungi can form heterokaryons during the sexual reproduction or asexual (vegetative) growth. The formation of heterokaryons requires the development and differentiation of some reproductive structures. In heterothallic ascomycetes, the formation of a sexual heterokaryon is mediated by alleles of a mating-type locus (MAT genes). The formation of an asexual heterokaryon is a result of a fusion of somatic cells under laboratory conditions and it is revealed by complementarity of "dietary" or pigment mutation (Leslie and Yamashiro, 1997). Loci for idiomorphic types of populations (MAT1-1 and MAT1-2) in species of the *Fusarium*

genus are not functionally related to *vic* genes, i.e. the sexual development of the fungus is not related to the expression of vegetative incompatibility (Klein and Correll, 2001). For instance, fertility of the *G. fujikuroi* species complex was not reduced by laboratory mating in which conidia (male gametes) differing in *vic* loci were used. On the other hand, in the case of two strains of *F. verticillioides*, it was experimentally proven that they belonged to the same VCG, but differed in the idiomorphic type of population. Klein and Correll (2001) considered it as a proof that there were no typical biochemical stages in the formation of sexual and vegetative heterokaryons.

The study of vegetative compatibility is important for many reasons. In some cases, this is an objective test for genetic similarity or dissimilarity because vegetative compatibility in ascomycetes, with better-characterised genetic systems, is a result of the actions of alleles at several distinct loci. The alleles at all *vic* loci must be identical for two strains to be compatible. Thus, the more closely related two isolates are genetically, the more likely they are to be vegetatively compatible. Therefore, VCG determination may reflect genetic similarities among strains of a species. A more important aspect of VCG is what it might imply about the reproductive strategy and population structure of a fungus. *F. oxysporum* is presumed to reproduce vegetatively. If this assumption is accurate, strains closely related by clonal descent should be within the same VCG because clonally derived strains would be isogenic, or nearly so, and, therefore vegetatively compatible, unless they happen to be vegetatively self-incompatible (Leslie, 1993). Additionally, strains within separate clonal lineages and in different VCGs might then be isolated genetically due to vegetative incompatibility. Clonal reproduction and the lack of meiotic recombination would greatly limit or preclude reassortment of genes for heterokaryon incompatibility. Hence, a population structure of *F. oxysporum* consisting of distinct clonal lineages corresponding to distinct VCGs suggests an absence of genetic recombination between members of these VCGs/lineages (Kistler, 1997). *F. oxysporum* is a cosmopolitan pathogenic fungus causing wilt diseases and cortical rots in more than 100 economically important plant hosts. Genetic diversity within *F. oxysporum* is often categorised by means of VCGs in laboratories worldwide. Detailed studies have shown that strains belonging to the same VCG typically have very similar or identical multi-locus haplotypes and belong to the same clonal lineage. Consequently, VCGs can be good predictors of genetic similarity, clonal lineage, or both (Kistler et al., 1998).

AUXOTROPHIC MUTANTS

Mutations often inactivate a biosynthetic pathway of a microorganism, and frequently make it unable to grow on a medium lacking an adequate supply of the pathway's end product. Based on this principle, microorganisms are classified as prototrophic and auxotrophic. Prototrophic organisms (wild type) have the same nutritional requirements as those of their ancestors. Auxotrophs are mutants for particular nutrient synthesis pathway enzymes. They can grow only on an enriched medium that provides the particular nutrient that the mutants cannot metabolise on their own (Amrita Vishwa Vidyapeetham Virtual Lab, 2012).

Heterokaryosis and mitotic recombination are an alternative to sexual recombination for genetic interaction among fungi. An increased frequency of somatic mitotic recombination has been observed after actions by certain mutagens. Vegetative compatibilities are generally determined by complementary auxotrophic strains or strains formed by spontaneous mutation during nutrition that are capable of forming a prototrophic heterokaryon. Heterokaryosis and vegetative compatibility have been observed in many fungi by using auxotrophic *nit* mutants (nitrate non-utilising mutants) that are considered to be exceptional genetic markers (Correll et al., 1987; Bowden and Leslie, 1992). An advantage of using *nit* mutants is their rapid isolation without employment of mutagenic treatments (Puhalla, 1985).

Most fungi can utilise nitrate as a nitrogen source by reducing it to ammonium (NH_4) via nitrate reductase and nitrite reductase. Some fungi, such as *Basidiomycetes, Saprolegniaceae* and *Blastocladiales*, are unable to utilise nitrate and cannot synthesise nitrate reductase (Correll et al., 1987). Cove (1976) has discovered that mutants not resistant to chlorate ($KClO_3$) are also *nit* mutants. Chlorate, a nitrate analogue, has been used for studying hetorokaryosis in *Fusarium* and nitrate assimilation in fungi, bacteria, algae and plants. *Nit* mutants are recovered from chlorate-resistant sectors that have intensive growth on the minimal medium amended with chlorate. The reduction of chlorate to chlorite by nitrate reductase can result in chlorate toxicity in the mentioned organisms. Chlorate-sensitive strains of fungi can reduce nitrate to nitrite, while chlorate-resistant strains cannot. Generally, the development of chlorate-sensitive strains is limited by the inner reduction of chlorate to toxic chlorite (Correll at al., 1987). The growth of wild type isolates is limited by chlorate (Puhalla, 1985), probably because chlorate is reduced by nitrate reductase to highly toxic chlorite.

Nitrate reductase in chlorate-resistant parts of the colony is prevented due to mutations in gene loci responsible for reductase of this enzyme. *Nit* mutants are usually incapable to reduce chlorate to chlorite due to lesions at one or several loci that control nitrate reductase and therefore express resistance to chlorate (Correl et al., 1987; Liu and Sundheim, 1996).

Nit mutants can be divided into three phenotypic classes. These classes presumably reflect mutations at a nitrate reductase locus (*nit1*), a nitrate-assimilation pathway-specific regulatory locus (*nit3*) and loci (at least five) that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (Correll et al., 1987). The combined abilities of the different classes of *nit* mutants to use various nitrogen sources can be used as a means to distinguish them as well. Wild-type strains can utilise all of the nitrogen sources, but are sensitive to chlorate. *Nit1* strains can grow on all of the media except for those with nitrate as the sole nitrogen source, on which they develop little aerial mycelium. *Nit3* mutants can grow on all media except for those with nitrate or nitrite as the nitrogen source, on which they develop without abundant aerial mycelium. NitM mutants can grow with abundant aerial mycelium only on media with nitrite or ammonia as the nitrogen source. *Crn* mutants can grow with abundant mycelium on all of the media and are both chlorate-resistant and nitrate-utilising. (Leslie and Summerell, 2006).

DETERMINATION OF VCGs

A well-developed procedure by means of *nit* mutants isolation from fungal pure cultures and by their pairing is applied to determine VCGs (Leslie, 2003). In this procedure, special attention is paid to preparation of specific nutrient media.

The following media are used in VCG tests: basal medium (BM), minimal or nitrate medium - basal medium with 2 g $NaNO_3$ 1000 ml^{-1} (MM or BM+NO_3), minimal medium with chlorate (MMC or MM+L-asparagine+$KClO_3$), ammonium medium - basal medium with 1 g of ammonium nitrate 1000 ml^{-1} (BM+NH_4), nitrite medium - basal medium with 0.5 g of $NaNO_2$ 1000 ml^{-1} (BM+NO_2) and hypoxanthine medium - basal medium with 0.2 g of hypoxanthine 1000 ml^{-1} (BM+hypoxanthine). The basal medium (BM) is prepared as follows (per litre of distilled water): 30 g of sucrose, 1 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 10 mg of $FeSO_4 \cdot 7H_2O$, 20 g of agar and 0.2 ml of a trace element solution. The trace element solution contained (per 95 ml of distilled water): 5 g of citric acid, 5 g of $ZnSO_4 \cdot 7H_2O$, 1 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.25 g of $CuSO_4 \cdot 5H_2O$, 50 mg of $MnSO_4 \cdot H_2O$, 50 mg of H_3BO_3 and 50 mg of $NaMoO_4 \cdot 2H_2O$ (Puhalla, 1985).

The procedure for VCG determination or VCG method encompasses several linked stages (Lević, 2008):

a) identification of *nit* mutants on the minimal medium (MM) supplemented with L-asparagine and chlorate;

b) determination of *nit* mutant phenotype on the basal medium with various nitrogen sources added, and determination of the class (type) of a mutant;

c) identification of complementary *nit* mutants (*nit1* x NitM or *nit1* x *nit3*);

d) interpretation of results.

Identification and determination of *nit* mutant phenotype

In order to identify *nit* mutant phenotype it is necessary to purify strains by a procedure of developing single spore cultures cultivated on potato dextrose agar at 25°C for 5-7 days. Fragments of single spore cultures should then be subcultured on the minimal medium supplemented with chlorate and cultured at 25°C for two weeks. Depending on strains or species, the concentration of chlorate varies from 0.5% to 6%, but mostly from 1.5% to 3%. If a sufficient number of *nit* mutants is not isolated, the concentration of potassium chlorate will increase, and then L-asparagine should be replaced with threonine or L-asparagine and potassium nitrate should be omitted from the basal medium, which has proved to be the most efficient change. Three to four fragments are placed in each of large Petri dishes (100.0 x 15.0 mm) in the form of equilateral triangle or rectangle. The number of fragments can be greater (e.g. 6) if the development of strains is weak. However, if several fragments are subcultured in each Petri dish it is recommended to check them more often, due to a risk of rapidly growing sectors covering the sectors that grow more slowly (Figures 1 and 2). The number of Petri dishes per isolate should be 10 to 15. All sectors are transferred to the minimal medium supplemented with nitrate as a nitrogen source, and if the formed mycelium is thin and transparent, then it is the *nit* mutant (Figures 1 and 2). If the formed mycelium is abundant or has a wild type morphology, it is the *crn* mutant. To determine the physiological phenotype of *nit* mutants, fragments

of the mycelium, developed on the minimal medium, are transferred to media with different nitrogen sources (ammonium, nitrate, nitrite and hypoxanthine) and grown at 25°C for 3-4 days. The growth rate of mutants is evaluated in comparison to the wild-type parental strain, and scored as "+" if it is similar to the wild type (abundant aerial mycelium) or as "-" if the growth is weak and aerial mycelium is not formed (Table 1). All strains with abundant mycelial growth on nitrate medium are rejected.

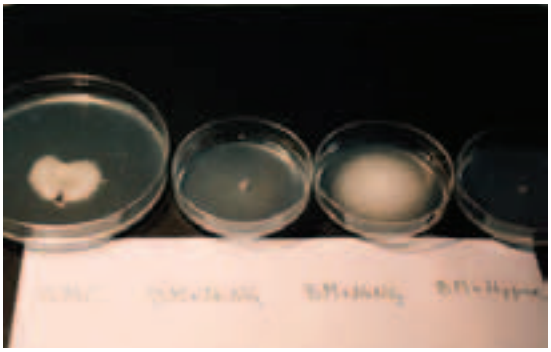

Figure 1. *F. oxysporum* - Chlorate-resistant sector on minimal medium with chlorate (MMC) and a phenotype of NitM mutant on basal medium supplemented with nitrate (BM+$NaNO_3$), nitrite (BM+$NaNO_2$) and hypoxanthine (BM+ hypoxanthine) (Krnjaja, 2005)

Figure 2. *F. oxysporum* - Chlorate-resistant sector on minimal medium with chlorate (MMC) and a phenotype of *nit1* mutant on basal medium supplemented with nitrate (BM+$NaNO_3$), nitrite (BM+$NaNO_2$) and hypoxanthine (BM+ hypoxanthine) (Krnjaja, 2005)

Based on the development of *nit* mutants on different nitrogen sources, they can be classified into the following classes or types (Table 1):

a) *nit1* mutants - nitrate-nonutilising mutants;

b) *nit3* mutants - nitrate- and nitrite-nonutilising mutants;

c) NitM mutants - nitrate- and hypoxanthine-nonutilising mutants.

Table 1. Physiological phenotype of chlorate resistant mutants

Strain Type	Growth on nitrogen sources[a]			
	NH_4	NO_3	NO_2	hypoxanthine
nit1	+	–	+	+
nit3	+	–	–	+
NitM	+	–	+	–
crn	+	+	+	+

[a]Growth on basal medium with various nitrogen sources; + = typical wild-type growth, - = thin growth with no aerial mycelium

Identification of complementary strains and VCGs

Vegetatively compatible *nit* mutants can complement one another in a following way: anastomosis is formed between two hyphae, genetic material is exchanged and cells with several nuclei or heterokaryons are formed. The phenotypical recognition of the formation of heterokaryons is easy because a dense white aerial mycelium is formed in the area where colonies come in contact (Correll et al., 1987) (Figure 3). The complementation test of *nit* mutants is performed on the minimal medium (MM) that contains nitrate as a nitrogen source. The pairing of two strains occurs on this medium - one strain is of the *nit1* type and the other is of the NitM type or *nit3*, if NitM was not isolated from the same strain of the species.

Figure 3. *F. oxysporum* - heterokaryon compatibility in the form of visible heterokaryon (abundant aerial mycelium) and heterokaryon incompatibility (without aerial mycelium) between fragments of *nit* mutants (Krnjaja, 2005)

Tests of *nit* mutant pairings can be done in Petri dishes or 24-96 tissue culture well plates. Incubation of paired mutants is performed at 25°C for 7-15 days. Mutants are frequently paired in Petri dishes - four mutants per dish (100.0 x 15.0 mm). The formation of heterokaryons in all four combinations is an indicator that the strains

A and B are of the same VCG, while their presence only in the combinations *nit1*A x NitMA or *nit1*B x NitMB indicates that they belong to different VCGs (Puhalla, 1985; Corell et al., 1987; Leslie, 1993; Leslie and Summerell, 2006; Lević, 2008). Self-compatibility in complementation tests indicates virulence and pathogenicity of the isolate. Self-incompatible strains or heterokaryon self-incompatible (HIS) strains are not virulent and therefore there inclusion into complementation tests is not necessary because they will not express a positive reaction in forming a heterokaryon (Puhalla, 1985).

Interpretation of results

The fundamental principle in identification of *nit* mutants is based on their resistance to chlorate and nitrate, and results from their inability to reduce nitrate to toxic nitrite, which is analogous to an impossibility for reduction of chlorate to toxic chlorite. Although *nit* mutants are auxotrophs they will grow unrestrictedly on the minimal medium (Puhalla, 1985). Their radial growth is comparable to the wild-type strains but the development of aerial mycelium is very weak. Even though *nit* mutants are not genuine representatives of wild-type strains, due to their inability to utilise nitrogen, they have some traits similar to those of the wild-types, such as growth, pathogenicity, resistance to benomyl, survival, etc. (Takehara et al., 2003).

The mutants *nit1*, *nit3* and NitM may be used to form hetorokaryons, while *crn* mutants have to be identified and then rejected. After pairing of mutants that belong to different classes, heterokaryons are either formed if the mutants are of the same compatible group or not formed if they belong to different VCGs.

Some field strains are incapable of forming heterokaryons. Correll et al. (1987) explained in detail the occurrence of a wild-type colony on the minimal medium, originating from chlorate-resistant sectors. Colonies resulting from each microconidium from chlorate-resistant sectors either remained thin on the minimal medium (typical of *nit* mutant morphology) or had abundant aerial mycelium on the minimal medium (typical of wild-type morphology). The analysis of microconidia indicated that the chlorate-resistant nitrate utilising sectors could be homokaryotic or heterokaryotic. Individual microconidia from homokaryotic sectors had a wild-type morphology on the minimal medium and presumably were mutants that were both chlorate-resistant and able to utilise nitrate (*crn* mutants). Microconidia recovered from heterokaryotic sectors were often a mixture of *nit* mutant conidia, wild-type conidia and/or *crn* mutant conidia (Correll et al., 1987).

Strains with growth similar to the wild-type strains are either wild-type isolates that are not tested or *crn* mutants able to utilise nitrates and chlorate-resistant. This type of mutants is a problem in VCG analyses. Generally, these mutants are rejected because they cannot form heterokaryons on the minimal medium. *Nit* mutants are not usually purified to single spore. However, unusual or doubtful results require single spore strains of *nit* mutants (Leslie, 2003).

The usefulness of *nit* mutants is great because they can be used to study similarities and dissimilarities of strains for vegetative compatibility worldwide. These techniques, coupled with virulence tests and pesticide toxicity tests, should provide valuable information on the genetic diversity of natural populations of this ubiquitous soil inhabitant (Lević, 2008).

CONCLUSION

Virulence has been and no doubt will continue to be a very useful property for characterising the diversity of strains within one population of fungi. Vegetative compatibility is a second property utilizable for identification of diversity among strains within one population of fungi, especially as strains with similar pathogenicity belong to the same VCG.

The strategy for population analysis by means of VCGs will depend on how genetic variation for *vic* loci will spread in the population. If a population is primarily clonal, or if some clonal haplotypes are important from the aspect of diseases or some other perspective, than VCG analysis can be quite useful once the correlations between VCG and some fungal traits have been confirmed. In populations in which genetic modifications and recombination occur, VCG analysis will provide information sufficient only for observing that strains are different. Generally, VCG studies of genotypically diverse populations should be avoided.

The system of vegetative compatibility-incompatibility is a very useful method in studies of populations dynamics of pathogenic and non-pathogenic fungal strains. This system is employed to identify new races in particular geographic crop regions. The system of vegetative compatibility-incompatibility assessment is easy, rapid, cheap and macroscopic score is used in complementation tests (pairing tests).

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Primena testova vegetativne kompatibilnosti za identifikaciju biodiverziteta fitopatogenih gljiva

REZIME

Vizuelna ocena fenotipa koja se izvodi kada se dva izolata ili soja jedne vrste gljive gaje u združenim kulturama na specifičnim podlogama, poznata kao test vegetativne ili heterokarionske kompatibilnosti ili nekompatibilnosti, omogućava identifikaciju klonova gljive i njihovu klasifikaciju prema filogenetskim grupama. Hife izolata koje imaju identične alele kod svih *vic* lokusa mogu da anastomoziraju u oblik vidljivog heterokariona. Izolati koji dele kompatibilne lokuse i mogu da anastomoziraju jedni s drugima pripadaju subpopulaciji označenoj kao vegetativno kompatibilna grupa (VCG) koja je genetski odvojena od drugih vegetativno kompatibilnih grupa (VCGs). Svaka VCG je specifična prema biljci domaćinu ili srodnoj grupi domaćina i može ili ne mora biti virulentna na drugim domaćinima.

Vegetativna kompatibilnost može biti utvrđena na različite načine, ali se najčešće koriste komplementarni auksotrofni sojevi, ili sojevi koji su nastali spontanom mutacijom tokom ishrane, a koji su sposobni da obrazuju prototrofni heterokarion. Smatra se da su *nit* mutanti izvrsni genetički markeri za utvrđivanje vegetativne kompatibilnosti i grupisanje sojeva ili klonova jedne vrste gljive u iste ili različite VCGs. Nesposobnost da se odredi stepen srodnosti korišćenjem VCGs, već samo da li su izolati isti ili ne, ograničava tipove analiza koje bi mogle biti postavljene. VCGs su najefikasnije kada se koriste za utvrđivanje prisustva specifičnog soja u populaciji.

U radu je dat pregled značaja fenomena vegetativne kompatibilnosti, kao jedne veoma značajne genetičke osobine kod askomiceta kojom se jedna subpopulacija može identifikovati kao posebna genetička grupa. Isto tako, dat je detaljan opis postupka za izolaciju, identifikaciju i utvrđivanje fenotipa *nit* mutanata, kao i za identifikaciju komplementarnih sojeva i VCGs.

Ključne reči: Vegetativna kompatibilnost; fitopatogene gljive; genetički diverzitet; VCGs