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# RELATIONSHIP BETWEEN PATHOGENICITY, RACE AND VEGETATIVE COMPATIBILITY GROUPING AMONG ALGERIAN POPULATIONS OF *FUSARIUM OXYSPORUM* F.SP. *PISI* CAUSING PEA WILT

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## RÉSUMÉ

*Fusarium oxysporum* f.sp. *pisi* (Linf.) Snyder et Hansen (FOP), est un agent pathogène significatif et destructeur du Pois en Algérie. Dans la présente étude, cinquante isolats *de Fusarium oxysporum* f.sp. *pisi* l'agent causal du flétrissement vasculaire du Pois (Pisum sativum), collectés dans différentes régions de l'ouest Algérien et représentants quatre races de l'agent pathogène, ont été analysés pour leur virulence. L'incidence du flétrissement vasculaire variait de 6,66% à 88,33% sur la variété très sensible (Little Marvel). Parmi lesquelles, 21isolats appartenant à quatre races de FOP et un isolat de FO non pathogène ont été analysés pour la compatibilité végétative afin de révéler la structure génétique de la population et de vérifier la fiabilité de la méthode pour l'identification des races physiologiques de FOP. Les résultats obtenus ont montré que les isolats de FOP pouvaient être classés en quatre groupes principaux de compatibilité végétative VCGs correspondant aux races 1, 2A, 2B et 5. Les isolats de la race 6 sont classés dans le même VCG que la race 1. A notre connaissance, c'est la première étude réalisée sur le sujet en Algérie

Mots-clés : *Fusarium oxysporum* f.sp. *pisi*, pathogénicité, races physiologiques, groupe de compatibilité végétative (VCG), l'ouest Algérien.

ABSTRACT: *Fusarium oxysporum* f.sp. *pisi* (Linf.) Snyder and Hansen (FOP), is a significant and destructive pathogen of pea in Algeria. In the present study, fifty isolates of *Fusarium oxysporum* f.sp. *pisi* the causal agent of pea (Pisum sativum) wilt, collected from different parts of western Algeria and representing four races of the pathogen, were analyzed for virulence. The wilt incidence ranged from 6.66% to 88.33 % on a highly susceptible cultivar (Little Marvel). Among which twenty-one isolates belonging to four races of FOP and one nonpathogenic FO isolate were analyzed for vegetative compatibility in order to reveal the genetic structure of the population and to check the reliability of the method for the identification of physiological races of FOP. Obtained results showed that the FOP isolates could be classified into four main vegetative compatibility groups VCGs that corresponded to races I, 2A, 2B and 5. The race 6 isolate, fell into the race 1 VCG. To our knowledge, this is the first study in Algeria.

Keywords: *Fusarium oxysporum* f.sp. *pisi,* pathogenicity , physiologic races, vegetative compatibility group (VCG),western Algeria.

## INTRODUCTION

*Fusarium oxysporum* f.sp. *pisi* (FOP) the causal organism of pea wilt, occurs as a number of races identified by tests on differential hosts of pea. So far, four races, 1, 2, 5 and 6, have been distinguished Races 1, 2 and 6 have been reported in Europe, while all four races are found in the U.S.A (Haglund & Kraft, 2001) and in Algeria (Merzoug et al., 2014).The pathogenicity test to determine the race of an isolate is consuming time and greatly affected by environmental conditions. In the absence of a sexual stage another way of grouping Fusarium isolates was proposed, it is by their ability to form heterokaryons by anastomosis. A Vegetative compatibility (VCG) in fungi is a genetic-trait controlled by vic or het loci and identical alleles at each loci must be present in two compatible hyphae before anastomosis takes place (Leslie, 1993). Vegetatively compatible isolates of a fungal species are placed in the same VCG. Isolates within the same VCG often share common biological, physiological, and pathological characteristics. VCGs are identified using nitrate no utilizing (nit) auxotrophic mutants that show thin but expansive growth on minimal medium with nitrate as sole nitrogen source. Isolates are considered vegetative compatible when complementing nit mutants anastomose and produce wild type growth (Puhalla, 1985).That exchange of genetic material would be limited to compatible isolates within a VCG and so each VCG represents a genetically isolated population. Vegetative compatibility has been used to classify isolates of *F. oxysporum* belonging to distinct formal specials (Klein et al., 2005); races within a special form (Elena & Pappas, 2006); special forms and/or races within or between different geographical origins (Diprimo et al., 2002; Pasquali et al., 2005) and non-pathogenic isolates ( Edel-Hermann et al., 2004).VCGs has been use to distinguish between nonpathogenic and pathogenic populations on the same host species. In general, pathogenic isolates of F. oxysporum in the same VCG are assumed to be associated with the same clonal lineage, even if they are geographically isolated (Leslie, 1993). However, isolates belonging to different VCGs can also belong to the same clonal lineage (Baayen et al, 2000). Several formae speciales of F. oxysporum have been characterized by VCG analysis (Lori et al. , 2004; Abo et al., 2005), as well as by RAPD analysis (Álves-Santos et al. 2007; Bayraktar et al., 2008; Baysal et al,. 2010) and several studies have combined both markers (Vakalounakis et al,. 2004 ; Nagarajan et al.,,2006). This technique is appropriate for developing countries with inadequate facilities for molecular work and can rapidly determine genetic groups of many fungal pathogens and their relation to pathogenicity (Cumagun et al., 2008).The objective of this work was to determine VCGs in a collection of 21 isolates of different pathogenic races *F. oxysporum* f.sp. *pisi* in different geographical origins in four different agro-climatic zones in western Algeria (Coastal plains, Interior plains, the High plateaus, and the Sahara) and compatibility between pathogenic and non-pathogenic *F. oxysporum* isolates obtained from asymptomatic pea rhizosphere.

## MATERIALS AND METHODS

### Fungal isolates

The study was conducted with 50 isolates representing the 4 races of FOP collected from different regions of western Algeria (Figure1, Table1). Pathogenicity tests were performed on the standard pea differentials cultivars. The inoculum production and inoculation method have been previously described (Merzoug et al., 2014). Twenty-one isolates of different pathogenic races F. oxysporum f.sp. *pisi* and one non-pathogenic isolate (np) are selected for vegetative compatibility test. The choice of this population is based on pathogenicity, races and areas. The isolates were maintained as single-spore colonies in tubes with Potato-Dextrose-Agar (PDA), at 4°C. The isolates selected for the test are listed in Table 2.

### Vegetative compatibility tests

### Culture media

The media used in the vegetative compatibility study were PDA with 1.5% chlorate (PDC), salt Minimal Medium with 1.5% chlorate (MMC), minimal medium (MM) with nitrate, chlorate, nitrite and hypoxanthine added according to Puhalla (1985) and Correll et al., (1987).

Determination of VCGs

### Selection, characterization and storage of nit mutants

VCGs were determined through the complementation of nitrate-nonutilizing (nit) mutants as a visual indicator of heterokaryon formation (Puhalla 1985). Generation, isolation and characterization of nit mutants were carried out according to the methodology of Correll et al. (1987). The nit mutants were selected by Puhalla's method (Puhalla, 1985). For this, 2 mm PDA blocks with fungal mycelium were

transferred to 9 cm plates that contained PDA with 1.5% chlorate ( PDC) or a salt Minimal Medium with 1.5% chlorate (MMC) (Puhalla,1985), and were incubated at 25°C for 1 to 3 weeks. Fast growing sectors were transferred to Minimal Medium(MM) and whose whith grew as fine and expanding colonies, without aerial mycelium, were considered to be nit mutants. In cases where we were not able to select mutants under the conditions described above, we have introduced some modifications in order to increase the chlorate concentration in MMC or PDC up to 5%, or two cultures consecutive on MMC (1.5%) chlorate. Changes are inspired in the methodology described by Nogales- Moncada et al., (2009).

### Phenotype identification

Nit mutants were grown on basal medium amended with different nitrogen sources: sodium nitrate, sodium nitrite and hypoxanthine. Petri dish cultures were incubated under the conditions described and examined after 5 days for mutant identification. On the basis of an ability to grow on the different nitrogen sources, three types of mutants were easily identified. nit-1 mutants were mutated in a structural locus of nitrate reductase. Those mutants did not grow with nitrate as the only nitrogen source, but they did grow in the presence of nitrite or hypoxantine. nit-3 mutants were mutated in a locus which was probably involved in the regulation of both nitrate reductase and nitrite reductase. Consequently, these mutants did not grow with nitrate or nitrite as the only nitrogen source, but they did grow in the presence of hypoxanthine. Lastly, Nit-M mutants were mutated in one of the loci which were involved in the synthesis of the molybdenum co-factor, which is necessary for both the reduction of nitrate and the hydroxylation of hypoxanthine. Those mutants cannot grow on nitrate or hypoxanthine as the only nitrogen source, but they will grow on nitrite (Correll et al., 1987).

### Complementary tests

Vegetative compatibility was determined on the basis of the formation of heterokaryons in the contact area between the two colonies, as shown by the growth of aerial mycelium similar to that of the wild colony. A portion of the Nit-M colony was placed in the middle of the culture dish containing MM, and one portion each of nit-1 and nit-3 mycelium from other strains was placed at an equal distance from the Nit-M colony. Complementation between nit-1 and nit-3 mutants occurs less frequently than complementation between one of these mutants with a Nit-M mutant. Each Nit-M was paired with a nit-1 and a nit-3 from other strains in all possible combinations. All pairings were replicated twice. The complementation test was considered: - negative, when there was no proto-trophic growth in the mycelial line of contact; - weak, when interaction became evident by the appearance of a thin, sometimes non-continuous, zone of prototrophic growth with very little aerial mycelium and - strong, when a dense line of prototrophic growth with abundant aerial mycelium was obtained. Weak and strong reactions were taken as evidence of compatibility. Training the heterokaryon, visible by the appearance of a thick, dense aerial mycelium, is noted after 5, 10, 15, 25 days and up to 4 weeks.

Statistical analysis: The results were subjected to statistical analysis. All the collected data were submitted to ANOVA analysis using Statistica software v. 5.5 (Statsoft, Ed'99) and Biostat.2009 and the significance of differences among treatments was recorded at $p<0.05$. Multiple comparisons of the means were conducted according to the Newman–Keuls test at $p<0.05$.

## RESULTS

Results of percentage wilt incidence of 50 isolates of *Fusarium oxysporum* f.sp. *pisi* collected from different parts of western Algeria and representing four races of the pathogen ranged from 6.66% to 88.33 % on highly susceptible cultivar (Little Marvel) (Table 1), a partial account of this work has previously been published (Merzoug et al., 2014).

### Isolation, characterization and storage of nit mutants

The medium PDC that proved most favorable to the growth of mutants sectors is select .Isolation of chlorate-resistant mutants and phenotypic diversity of nit mutants were obtained from all FOP isolates, but there was considerable variation in their recovery rate. Whenever possible, at least two different nit mutants, preferably a nit 1 and a Nit M, were selected and stored as indicated in Materials and Methods.

### Vegetative compatibility tests

The results of the characterization of 343 chlorate-resistant mutants (Tab.2), indicate that in 180 (52.47%) of them are nit1, 106 (30.9%) Nit M and 57 (16.41%) nit 3. The overall distribution of three types of nit mutant according to the races, shows that the Race1 forms the greatest number of

mutant or a total de186 (54.22%), which represents more than half of all the mutants followed by race 6 with 65 (18.95%) . The highest number mutants nit 1 is obtained from race 1 with 99 (55%). Race 2 presented the number of mutant nit 3, the low with 7 (12.28%) (Tab.3).

**Table 1.** Geographic origin, races and wilt incidence of *Fusarium oxysporum* f.sp. *pisi* isolates.

| N° | Geographic origin | Departments | Isolates codes | Pathogenicity | |
|---|---|---|---|---|---|
| | | | | Wilt incidence (%) | Races |
| 1 | Coastal plains | A. Temouchent | Fop A1 | 58.33 (b) | 1 |
| 2 | | A. Temouchent | Fop A2 | 56.66 (b) | 1 |
| 3 | | A. Temouchent | Fop A16 | 25.66 (d) | 6 |
| 4 | | A. Temouchent | Fop A18 | 21.00 (d) | 2 |
| 5 | | A. Temouchent | Fop A19 | 26.66 (d) | 1 |
| 6 | | A. Temouchent | Fop A21 | 36.33 (c) | 5 |
| 7 | | A. Temouchent | Fop A22 | 41.33 (c) | 6 |
| 8 | | Mostaganem | Fop M42 | 36.66 (c) | 5 |
| 9 | | Mostaganem | Fop M43 | 38.00 (c) | 2 |
| 10 | | Mostaganem | Fop M44 | 45.00 (c) | 1 |
| 11 | | Mostaganem | Fop M45 | 40.33 (c) | 1 |
| 12 | Interior plains | Tlemcen | Fop TL24 | 25.66 (d) | 6 |
| 13 | | Tlemcen | Fop TL27 | 56.33 (b) | 1 |
| 14 | | Tlemcen | Fop TL28 | 60.00 (b) | 6 |
| 15 | | Tlemcen | Fop TL29 | 71.66 (b) | 1 |
| 16 | | Tlemcen | Fop TL31 | 69.66 (b) | 1 |
| 17 | | Tlemcen | Fop TL32 | 69.00 (b) | 1 |
| 18 | | Chlef | Fop C20 | 78.00 (b) | 1 |
| 19 | | Chlef | Fop C21 | 63.33 (b) | 1 |
| 20 | | Relizane | Fop R17 | 60.00 (b) | 1 |
| 21 | | Relizane | Fop R26 | 70.00 (b) | 2 |
| 22 | | Relizane | Fop R27 | 46.66 (c) | 1 |
| 23 | | Relizane | Fop R28 | 43.33 (c) | 1 |
| 24 | | Relizane | Fop R29 | 83.33 (a) | 1 |
| 25 | | Relizane | Fop R31 | 66.66 (b) | 1 |
| 26 | | Relizane | Fop R32 | 63.33 (b) | 6 |
| 27 | | Mascara | Fop Ma6 | 60.66(b) | 2 |
| 28 | | Mascara | Fop Ma9 | 46.66 (c) | 2 |
| 29 | | Mascara | Fop Ma13 | 60.33 (b) | 1 |
| 30 | | Mascara | Fop Ma15 | 60.66 (b) | 2 |
| 31 | | Mascara | Fop Ma19 | 65.00 (b) | 1 |
| 32 | | Sidi Belabes | Fop Sb1 | 86.66 (a) | 1 |
| 33 | | Sidi Belabes | Fop Sb3 | 60.00 (b) | 2 |
| 34 | | Sidi Belabes | Fop Sb4 | 62.33 (b) | 1 |
| 35 | | Sidi Belabes | Fop Sb11 | 67.00 (b) | 1 |
| 36 | | Sidi Belabes | Fop Sb14 | 66.66 (b) | 1 |
| 37 | High plateaus | Tiaret | Fop T46 | 35.00 (c) | 1 |
| 38 | | Tiaret | Fop T47 | 40.00 (c) | 2 |
| 39 | | Tiaret | Fop T48 | 23.33 (d) | 1 |
| 40 | | Tiaret | Fop T49 | 50.00 (c) | 6 |
| 41 | | Tiaret | Fop T50 | 44.33 (c) | 1 |
| 42 | | Tiaret | Fop T51 | 39.00 (c) | 1 |
| 43 | | Tiaret | Fop T52 | 40.00 (c) | 1 |
| 44 | | Saida | Fop S56 | 88.33 (a) | 1 |
| 45 | | Saida | Fop S58 | 34.33 (c) | 1 |
| 46 | | Adrar | Fop | 6.66 ( e) | *np* |

| | | | | | |
|---|---|---|---|---|---|
| | | | Ad60 | | |
| 47 | Sahara | Adrar | Fop Ad67 | 88.33(a) | *2* |
| 48 | | Adrar | Fop Ad62 | 60.00 (b) | 2 |
| 49 | | Adrar | Fop Ad63 | 68.33 (b) | 1 |
| 50 | | Adrar | Fop Ad66 | 48.33 ( c) | 1 |

*np* – nonpathogenic *F. oxysporum*
the values with a common letter do not differ significantly at 5% level using the Newman–Keuls test.

**Table 2.** Number of each nit mutant type selected on characterization media in relation to geographic origin

| Isolate | Geographic origin | Department | Race | nit-1 | nit-3 | Nit-M | Total |
|---|---|---|---|---|---|---|---|
| A2 | Coastal plains | Ain Temouchent | 1 | 10(100%)[a] | 0(0%) | 0(0%) | 10 |
| A21 | | Ain Temouchent | 5 | 10(41.66%) | 8(33.33%) | 6(25%) | 24 |
| A22 | | Ain Temouchent | 6 | 8(66.66%) | 0(0%) | 4(33.33%) | 12 |
| M42 | | Mostaganem | 5 | 9(69.23%) | 0(0%) | 4(30.76%) | 13 |
| M44 | | Mostaganem | 1 | 11(45.83%) | 1(4.16%) | 12(50%) | 24 |
| Tl 24 | Interior plains | Tlemcen | 6 | 7(58.33%) | 0(0%) | 5(41.66%) | 12 |
| Tl27 | | Tlemcen | 1 | 13(86.66%) | 0(0%) | 2(13.33%) | 15 |
| Tl28 | | Tlemcen | 6 | 16(88.8%) | 0(0%) | 2(11.11%) | 18 |
| C20 | | Chlef | 1 | 6(60%) | 2(20%) | 2(20%) | 10 |
| C21 | | Cheliff | 1 | 6(54.5%) | 1(9%) | 4(36.3%) | 11 |
| R26 | | Relizane | 2 | 11(100%) | 0(0%) | 0(0%) | 11 |
| R28 | | Relizane | 1 | 2(20%) | 2(20%) | 6(60%) | 10 |
| Ma13 | | Mascara | 1 | 14(63.6%) | 0(0%) | 8(36.36%) | 22 |
| Ma15 | | Mascara | 2 | 7(35%) | 7(35%) | 6(30%) | 20 |
| Sb1 | | Sidi Belabes | 1 | 8(88.8%) | 1(11.1%) | 0(0%) | 9 |
| Sb11 | | Sidi Belabes | 1 | 12(46.15%) | 0(0%) | 14(53.84%) | 26 |
| T49 | High plateaus | Tiaret | 6 | 5(21.73%) | 16(69.56%) | 2(8.69%) | 23 |
| T51 | | Tiaret | 1 | 2(9.09%) | 4(18.18%) | 16(72.72%) | 22 |
| S58 | | Saida | 1 | 5(33.33%) | 5(33.33%) | 5(33.33%) | 15 |
| Ad62 | Sahara | Adrar | 2 | 6(60%) | 0(0%) | 4(40%) | 10 |
| Ad63 | | Adrar | 1 | 10(83.33%) | 0(0%) | 2(16.66%) | 12 |
| Ad60 | | Adrar | NP | 2(14.28%) | 10(71.42%) | 2(14.28%) | 14 |
| | | Total | 22 | 180(52.47%) | 57(16.41%) | 106(30.9%) | 343 |

*percentage of isolates in that group; np – nonpathogenic F. oxysporum

**Table 3.** Distribution of numbers and mutants (nit) percentage according to the different physiological races of *Fusarium oxysporum* f. sp. *pisi*

## Development of the pairs of mutants

When possible, a nit 1 and a Nit M mutant from each isolate were selected and paired in all possible combinations. The positive complementarity between different nit mutants resulted in the growth of aerial mycelium in the confrontation zone, resulting from the anastomosis hyphae matched mutant colonies. In this study, the line of fusion of the hyphae varied in its density and appeared 10-15 days after transfer of cuttings on the MM medium. It consists of a continuous growth line of a thick and abundant mycelium, or as weak aerial mycelium line dispersed and more or less dense or less defined line separates with a mycelium +/- flush that appears after 15 days up to a month (Fig1). The results of various comparisons between nit mutants complementary to a same isolate were self-compatible for all isolates tested except for two race 1 isolates(Sb1 and A2) and one race 2 isolate (R26) which was self-incompatible knowing that for the three isolates only the nit1 was obtained. The non-pathogenic isolate was self-compatible.

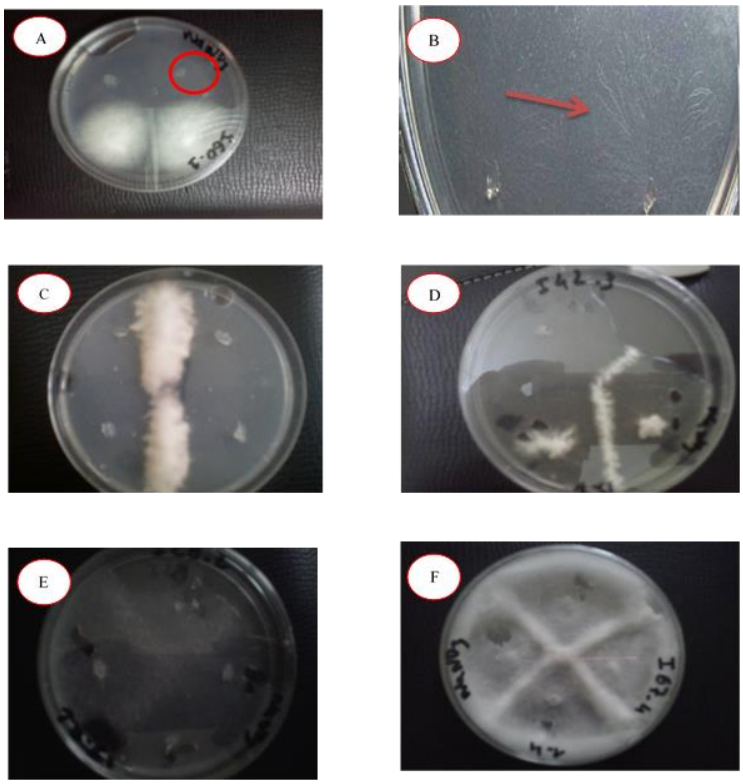


**Fig. 1**. Vegetative compatibility tests. A–B – growth of nit mutants on minimal medium (MM). The elliptical areas – the chlorate resistant sectors; the arrow development of thin mycelium. Pairing of nit mutants subcultured on MM; C – continuous growth line of a thick and abundant aerial mycelium; D – weak aerial mycelium line dispersed; E – less defined line separates with a mycelium +/– flush, that appears after a month; F – self-compatibility (np isolate Ad67).

| nit-mutant | R1 | R2 | R5 | R6 | NP | Total |
|---|---|---|---|---|---|---|
| nit-1 | 99(55%) | 24(13.3%) | 19(10.5%) | 36(20%) | 2(1.1%) | 180 |
| nit-3 | 16(28%) | 7(12.28%) | 8(14.03%) | 16(28%) | 10(17.5%) | 57 |
| Nit-M | 71(66.98%) | 10(9.43%) | 10(9.43%) | 13(12.26%) | 2(1.88%) | 106 |
| Total | 186(54.22 %) | 41(11.95%) | 37(10.78%) | 65(18.95% ) | 14(4.08%) | 343 |

## Vegetative compatibility group (VCG)

The analysis of results of the complementation test listed in (Table4) shows:

1- Race 2 isolates are classified into two subgroups, the Ma15 isolate was self-compatible but incompatible with all other isolates same with those of the race 2. Isolate R 26 is self-incompatible but it is compatible with the isolate Ad 62 ,these two isolates are distant geographical origin (internal plains and Sahara) for Ma 15 and R 26 which are incompatible thy have the same geographical origin.

2 -The race 5 isolates are classified in the same VCG. They are compatible with each other, they showed a slow complementation that occurred after month of confrontation with isolates of race 1 (Sb1, Ad 63, S58, C24 and A2). The only positive complementation is observed between M44 and M 42, which are from the same region (Mostaganem).

3- Race 1 isolates are classified in the same VCG. All isolates are self-compatible except Sb1 and A2 isolates were self-incompatible, which formed only nit1 that produce a negative or inconclusive complementation. Compatibility relationships within the race 1 VCG are not simple. The reaction of compatibility between isolates was variable. For example, Sb1 isolate is only compatible with the TL27 and weak reaction with R28, while TL27 is compatible with all isolates of race 1 except with S58. Ma13 is compatible with TL27 and not compatible with Sb1.

4- The race 6 isolates are classified in the same GCV that race1.The race 6 isolates were all self-compatible. Complementation was positive or partial between most race 6 isolates.

5 -The non-pathogenic isolate (NP)was self- compatible. It was compatible with race 1 and race 6 isolates and is incompatible with the R2 races and race 5 isolates.

There was no compatibility between the VCGs that comprised races 2A and 2B and any other VCGs. However, there was a weak interaction between some race1 and race 5 isolates, although these interactions only developed after at least 4 weeks incubation (Table 5).

The results obtained show that the FOP isolates from western Algeria can be classified into four main vegetative compatibility groups VCGs that corresponded to races I, 2A, 2B and 5. The race 6 isolate, fell into the race 1 VCG, and although compatibility was not complete with any race 1 isolate tested. In addition, after several weeks' incubation it also showed weak compatibility to some race 5 isolates. According to the procedure for determining vegetative compatibility group described by (Correll, 1991) and the systematic numbering proposed by Katan & Katan (1999). The VCGs FOP code number is 0070-0073.

## DISCUSSION

In this study, virulence and vegetative compatibility were analyzed for FOP isolates representing four races prevalent in the west of Algeria. The range of wilt incidence (6.33–88.33%) caused by the isolates showed variability in the virulence of the pathogen even on a single susceptible variety of pea. In the present work, the three types of mutant's nit whose highest percentage is the nit-1 followed by Nit-M and the lower the nit-3, without these differences may be related to a specific characteristic of isolates (geographical origin, race or virulence). According to Puhalla (1985), the frequency of nit1 mutants is higher than the frequency of the other types of nit mutants. The percentage of nit-3 is always lower (Belabid & Fortas, 2002). The study of the organization of 21 isolates in the formae speciales *pisi* from different geographical origins (west of Algeria) and belong to the 4 races of FOP has shown the existence of four VCG S, reflecting its genetic diversity. Such diversity has been reported for many formae speciales such as F. oxysporum radicis-lycopersici (Katan & Katan, 1999), ciceris (Nogales- Moncada et al., 2009) and batatas (Rodriguez-Molina et al. 2013). Comparing the VCG distribution and races distribution defined by means of differential hosts, showed that isolates belong to different races are included in the same VCG (Saabale & Dubey, 2014). This is the case of the isolates of the race 1 and 6 *F. oxysporum* f. sp. *pisi* which are in the same VCG, whereas isolates race 2 are included in two other VCG. This situation is found in other formae speciales for which the VCG-race relationships are complex. Indeed, it is known that more than one race may appear in the same VCG and the same race may contain more than one VCG (Correll, 1991). A similar degree of complexity has been noted in the *F. oxyspomrn* f.sp.*cubense* (Somrith et al., 2011), many of which belong to the same races and multiple VCG correspond to single race. It is the same at the *F. oxysporum* f. sp. *lycopersici* (Katan & Katan, 1999) and *F. oxysporum* f. sp. *melonis* (Elena& Pappas, 2006).

The results of the weak or partial vegetative compatibility observed between isolates of race 1 and 5, suggesting a high degree of similarity with isolates of race 1 than they are to those of race 2A and 2B. According to Whitehead et al., (1992), it seems likely that the race 5 and 6 derived from the race 1. The

greatest degree of intra-racial variation seen among race 1 isolates may suggest that this is the oldest race. The differences between races 1 and 5 compared to the similarity between races 1 and 6 would indicate that race 6 may have evolved much more recently than race 5. Race 2 is very different from races 5, 6 and 1 and may have a distinct evolutionary origin. Races 2A and 2B are clearly very closely related but the total lack of vegetative compatibility between them demonstrates that they are genetically distinct populations (Whitehead et al., 1992). It is possible that 2A and 2B are two separate races and might be distinguished by pathogenicity testing if the range of differential lines was extended; new line differential has been demonstrated recently for a sharper characterization race 2. F. oxysporum f. sp. phaseoli (Alves-Santos et al., 2012). Generally, in various formae speciales all isolates of the same VCG are compatible with each other. In addition, some combinations between the race 1 isolates produced weak or partial reactions. These differences in the intensity of crop interactions have been reported in F. oxysporum and could reflect the result of the action of alleles at different loci affecting vegetative compatibility (Nogales- Moncada et al., 2009). Moreover, some isolates in the VCG were incompatible; that is, two isolates might be vegetatively incompatible even though each is compatible with a third isolate. On vegetative compatibility in V. dahliae, Rataj-Guranowska et al. , (2016) have observed bridges between American testers VCG 2 and VCG 4, also between American and Duch testers and between subgroup testers VCG 2A and 2B.The occurrence of these bridging strains is well known in *F. oxysporum* (Vakalounakis & Fragkiadakis, 2004). Another difference between FOP and other formae speciales is demonstrated by the anomalies found in the pairings of race 1 isolates, The presence of two sub-groups is not explained by geographical factors since isolates from both Europe and North America are present in the two subgroups (Whitehead et al., 1992). The weak complementation found between some race 1 and race 5 isolates even though they are in separate VCGs is not unique. Somrith (2011) reported that bridging isolates were capable of forming heterokaryons with isolates in both of two closely related VCGs in *F. oxysporum* f.sp. *cubense*. According to the analysis of Whitehead et al., (1992) it may be possible for weak complementation to occur in FOP even when two isolates differ slightly at one or more loci. Such differences could come about for example if a series of missense alleles of a particular het gene were present in the population. Some combinations might lead immediately to compatibility whereas others either would not or only do so after a long time, depending on the nature and position of the amino acid change (Nogales- Moncada et al., 2009). This could explain both the variation found in race 1 and the weak compatibility between some isolates of race 1 and race 5. The studies undertaken by different molecular techniques have allowed to distinguish the races 1, 5 and 6 are closely linked and that race 2 is distinct (Okubara et al., 2005; Sharma et al., 2006).

In this study, the complementation between pathogenic isolates FOP and non-pathogenic isolate FO has been tested. The non-pathogenic isolate has been vegetatively compatible with some isolates of the race 1 and race 6, it was grouped in the same VCG as race 1 and 6, the group includes pathogenic and non-pathogenic isolates. According a study conducted by Nogales-Moncada et al., (2009) on pathogenic and nonpathogenic isolates which most of the non-pathogenic isolates were compatible with a yellowing-type low virulent *F. oxysporum* f.sp. *ciceris* isolate, thus suggesting the possibility of the existence of transition isolates between pathogenic and non-pathogenic populations. Vegetative compatibility testing supported that *F. oxysporum* f. sp. *betae* is polyphyletic and that pathogenic isolates cannot be differentiated from nonpathogenic *F. oxysporum* using vegetative compatibility (Webb et al., 2013). Little is known, however, about the ecological significance and the population dynamics of the non-pathogenic strains of *F. oxysporum*, particularly at the subspecies level. Differentiating strains among the non-pathogenic F. oxysporum isolates is very important; they could provide a means of identifying and characterizing the various subpopulations of *F. oxysporum*. No specific relationship was observed between pathogenicity ,VCGs and geographic origin of the isolates in this study. According to several previous work these results are in agreement with results from previous studies with other *Fusarium spp.* (Mohammadi & Mofrad, 2009; Mohammadi et al, 2012) and on various formae speciales of *F.oxysporum* (Belabid & Fortas, 2002).

**Table 4**. Vegetative compatibility relationships between race isolates of *Fusarium oxysporum* f. sp. *pisi*

| Race | isolat | Ma15 | Ad62 | R26 | M42 | A21 | T49 | TI28 | TI24 | A22 | Sb1 | Sb11 | TI27 | Ad63 | S58 | Ma13 | C20 | A2 | TI51 | M44 | R28 | C21 | Ad60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ma15 | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| R2 | Ad62 | | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | R26 | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| R5 | M42 | | | | + | + | - | - | - | - | - | - | - | +/- | +/- | - | - | +/- | - | + | - | - | - |
| | A21 | | | | | + | - | - | - | - | - | - | - | +/- | - | - | +/- | +/- | - | - | - | - | - |
| R6 | T49 | | | | | | + | + | +/- | +/- | - | - | + | +/- | +/- | + | - | + | - | + | - | + | + |
| | TI28 | | | | | | | + | +/- | + | - | + | - | - | + | +/- | - | +/- | - | + | +/- | +/- | +/- |
| | TI24 | | | | | | | | + | + | - | - | + | - | +/- | - | + | - | +/- | - | - | - | + |
| | A22 | | | | | | | | | + | - | - | - | +/- | - | - | + | - | + | + | - | - | - |
| R1 | Sb1 | | | | | | | | | | - | - | + | - | - | - | - | - | - | - | +/- | - | +/- |
| | Sb11 | | | | | | | | | | | +/- | - | - | - | + | +/- | - | - | - | +/- | - | + |
| | TI27 | | | | | | | | | | | | +/- | +/- | - | + | + | + | + | + | + | + | + |
| | Ad63 | | | | | | | | | | | | | + | +/- | - | + | - | + | + | + | +/- | + |
| | S58 | | | | | | | | | | | | | | + | - | + | - | + | + | +/- | - | + |
| | Ma13 | | | | | | | | | | | | | | | + | - | - | +/- | + | - | - | +/- |
| | C20 | | | | | | | | | | | | | | | | + | - | +/- | + | - | +/- | + |
| | A2 | | | | | | | | | | | | | | | | | - | +/- | + | +/- | - | +/- |
| | TI51 | | | | | | | | | | | | | | | | | | + | - | + | - | +/- |
| | M44 | | | | | | | | | | | | | | | | | | | + | + | + | +/- |
| | R28 | | | | | | | | | | | | | | | | | | | | +/- | - | + |
| | C21 | | | | | | | | | | | | | | | | | | | | | + | +/- |
| NP | Ad60 | | | | | | | | | | | | | | | | | | | | | | + |

+: Compatible interaction; -: incompatible interaction; +/-: partially compatible interaction.

**Table 5**. Correlation between vegetative compatibility groups (VCG) and races

| Race | R1 | R2 A | R2 B | R5 | R6 | NP |
|---|---|---|---|---|---|---|
| R1 | + | - | - | +/- | + | + |
| R2A | | + | - | - | - | - |
| R2B | | | +/- | - | - | - |
| R5 | | | | + | - | - |
| R6 | | | | | + | + |
| NP | | | | | | + |

"+" – compatible interaction; "–" – incompatible interaction; "+/–" – partially compatible interaction; np – nonpathogenic F. oxysporum

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