Short Communication

Prevalence of Viruses Infecting Autochthonous Grapevines in Tunisia

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ABSTRACT

Mahfoudhi, N., Harbi-Ben Slimane, M., Elair, M., Selmi, I., and Ben Hamda H. 2014. Prevalence of viruses infecting autochthonous grapevines in Tunisia. Tunisian Journal of Plant Protection 9: 111-118.

The incidence of virus infections was conducted in the grapevine germplasm collection at the *Institut National de la Recherche Agronomique de Tunisie*. In this grapevine collection, 162 different autochthonous cultivars were maintained, including numerous spontaneous ecotypes coming from different Tunisian grapevine growing regions. All accessions were sampled and analyzed by DAS-ELISA for the presence of Grapevine leafroll associated viruses 1, 2, 3 (GLRaV-1, -2, -3), Grapevine fanleaf virus (GFLV), Grapevine fleck virus (GFkV) and Arabis mosaic virus (ArMV), using commercial polyclonal antisera. Almost all the major grapevine-infecting viruses assayed, except for ArMV, were detected in the tested cultivars. Conversely, all the wild grapevine accessions were found to be free from the same viruses. Out of 141 cultivars submitted to DAS-ELISA, 40.4% were infected with at least one virus. GLRaV-3 was the prevailing virus (23.4%), followed by GLRaV-1 (19.6%), GFkV (9.2%), GLRaV-2 (4.2%), and GFLV (1.4%). Cultivars collected from northern regions (61.4%) were more infected than their homologues from southern regions (19.7%).

Keywords: Autochthonous grapevine, DAS-ELISA, incidence, Tunisia, viruses

Grapevine (*Vitis vinifera*) is one of the oldest and most important perennial crops in the world. In spite of the abundance of bio-archaeological, historical and genetic data, the origin, identity of ancient grapevine cultivars and mechanisms of domestication are still largely unknown. The cultivation and domestication of the grapevine appears to have occurred between the seventh and the fourth millennia before Christ (16). In Tunisia, grapevine has proven flexibility in adapting to different conditions. Although the greatest diversity of native varieties is found in the southern regions, wild ecotypes (*Vitis vinifera* subsp. *sylvestris*) are located mainly in the Northwest and Northeast (5). Several native grapevine genotypes, highly appreciated for their organoleptic characteristics and commercial potential, are still cultivated in Tunisia. However, their substitution by more productive new international cultivars is causing depletion and a rapid decrease in the

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Accepted for publication 03 November 2014

number of local genotypes (21). In order to stop this loss of genes and genotypes, *Institut National de la Recherche Agronomique de Tunisie* (INRAT) has invested heavily in the collection of threatened cultivars and their preservation in a germplasm collection, which today includes more than 160 Tunisian autochthonous varieties and wild ecotypes. However, the real number of varieties might be significantly lower, due to the numerous cases of synonymy often found for the same varieties in different regions. Over time, the cultivars have adapted to their growing areas characterizing them with their typical products. In any case, to now 55 distinct genotypes have been identified and characterized among 80 cultivated accessions from the collection (18). Perhaps due to its long history of cultivation, grapevine plays host to the largest number of pathogens that can detrimentally affect life span, fruit quality and yield. Till now, seventy different grapevine-infecting viruses from diverse taxonomic groups have been identified (13). Among virus diseases, the most important are grapevine degeneration and decline (caused by nepoviruses), leafroll, rugose wood and fleck (14). In Tunisia, several studies have been conducted on viruses infecting grapevine-introduced varieties; they have shown that the main viruses described elsewhere are present in Tunisian vineyards, including those associated with leafroll (9, 12), rugose wood and degeneration (1, 11, 19) grapevine diseases. However, sanitary status of Tunisian native cultivars has remained unexplored until now, which means that the neglect of this aspect can lead to the deterioration and lack of interest or even the abandonment of certain cultivars. Therefore, it becomes crucial to know the incidence and distribution of grapevine viruses in order to create appropriate sanitary measures for genetic resources preservation and propagation programs. On these premises, the aim of the present work was to focus the attention on the native Tunisian cultivars, for which the assessment of the sanitary status, with reference to virus diseases, and the selection of virus free cultivars were pursued.

A total of 162 accessions were collected from all the Tunisian regions and conserved in a germplasm collection established in the area of INRAT headquarters. Northern cultivars were collected from Rafraf, Kef, Balta, Bargou, Djebba, Baddar, Zaghouan, and Sbiba, while those from southern regions were collected mainly from Mahdia, Sfax, Kerkenah, Gabes, Djerba, Tozeur, Degueche, and Nafta. Spontaneous ecotypes were from northern mountainous regions and CapBon. Sampling of mature canes was performed in winter, from all the accessions. Each sample was represented by at least two mature canes taken from different parts of the plant. Samples included 71 southern cultivars, 70 northern cultivars and 21 wild ecotypes. All samples were tested by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for the presence of Grapevine leafroll associated viruses 1, 2, 3 (GLRaV-1, -2, -3), Grapevine fanleaf virus (GFLV), Grapevine fleck virus (GFkV) and Arabis mosaic virus (ArMV). Extracts were obtained from mature canes by macerating cortical scrapings in a mortar in the presence of extraction buffer (Tris-buffer, pH 8.2). DAS-ELISA was carried out in polystyrene microtitre plates, following Clark and Adams protocol (2). Commercial kits (Agritest-Bari, Italy) were used. Absorbance was recorded at 405 ηm using an automatic microplate reader (Multiskan Ascent, Labsystems

USA) and samples with absorbance readings equal or exceeding three times that of the healthy controls were considered positive.

Serological analysis showed that almost all major tested grapevine infecting viruses are present in the native grapevine cultivars, except for ArMV. Out of 141 cultivars screened by DAS-ELISA, 57 (40.4%) were infected with at least one virus. Among these 57 cultivars, 36.8% were mixed infected by at least two viruses and 63.2% were single infected. Since all wild grapevines (21 accessions) were free from the tested viruses, results presented hereafter concern only the cultivars. GLRaV-3 was the prevailing virus, with an infection rate of 23.4%, followed by GLRaV-1 (19.6%), GFkV (9.2%), GLRaV-2 (4.2%) and finally GFLV (1.4%) (Fig. 1). The two ampeloviruses GLRaV-1 and GLRaV-3 were detected in mixture in 4.5% of infected cultivars (14/57). Cultivars collected from northern regions showed an infection rate of 61.4% (43/70). Among infected cultivars, 9.5% (4/43) were infected by at least two viruses. Surprisingly, the cultivars collected from southern region were significantly less infected, since only 19.7% (14/71) were DAS-ELISA-positive and 71.3% (10/14) of them were single infected. The distribution of virus infection between the north and the south originating cultivars is shown in Fig. 2. The two ampeloviruses associated with leafroll disease, GLRaV-3 (35.7%) and GLRaV-1 (34.2%), were more prevalent in the north than in the south, where they respectively reached only 11.2 and 5.6%. GFkV, the third virus in terms of occurrence, was detected in 14.3% of northern cultivars and in 4.2% of southern ones. Similarly, GLRV2 was more frequent on northern (5.7%) than on southern cultivars (2.8%), while GFLV was scarcely represented (1.4%) in both regions. With a mean infection rate of 35.7% in the tested cultivars from north, GLRaV-3 reached the highest incidence in Zaghouan (80%) and Rafraf (77%). It was less present in the other regions, ranging from 20% in Balta to 33.3% in Sbiba, and totally absent in the cultivars from Kef and Baddar (Table 1). GLRaV-1 prevailed particularly in Sbikha (66.6%), Zaghouan (60%), Rafraf (55.5%), and Djebba (50%); it was less represented in Balta and Bargou (10 and 12.5%, respectively) and completely absent in Kef and Baddar as well as GLRaV-3. These two ampeloviruses (GLRaV-1 and GLRaV-3), both transmissible by mealybugs, were detected in mixed infections in Zaghouan (60%), Rafraf (38.3%), Sbiba (33.3%), and Djebba (14.28%) (Table 1). Occurring at a mean infection rate of 14.3%, GFkV was detected only in the cultivars from Baddar (75%) and Rafraf (33.3%). GLRaV-2 closterovirus was detected in 14.3% of the cultivars from Djebba and in 12.5% of the cultivars from Bargou and Kef, whereas it was absent in all the other northern regions. Regarding to GFLV nepovirus, it was detected in one cultivar from Rafraf (5.5%).The virus incidence in the northern regions was high (61.4%), with a peak at Rafraf where samples were totally infected (100%). An unsatisfactory sanitary status was disclosed also in Zaghouan (80%), Baddar (75%), Sbiba (66.6%), and Djebba (64.3%) (Table 1). The other regions had an infection rate less than 50% (40, 25 and 12.5% in Balta, Bargou and Kef, respectively). Being the most widespread virus (11.2%), GLRaV-3 was detected in almost all regions, except Kerkenah and Gabes. Recorded infection rate reached the highest value in Djerba (37.5%), followed by Degueche (20%),

Sfax and Nafta (12.5%), Mahdia (11.1%) and finally Tozeur (8.3%) (Table1). For GLRaV-1, Degueche was the most infected region (20%), followed by Sfax (12.5%), Gabes and Tozeur (8.3%) whereas the remaining regions being GLRaV-1 free. GFkV, GLRaV-2 and GFLV had an infection rate less than 5%. GFkV was detected in Sfax (12.5%), Gabes and Tozeur (8.3%), while GLRaV-2 only in Sfax and Djerba (12.5%). In southern regions, the sanitary situation was better than in northern regions. Samples from Kerkenah were free of all tested viruses. The highest infection rate was registered in Djerba (37.5%), followed by Tozeur and Sfax (25%), Degueche (20%) and Gabes (16.6%) (Table 1). Nafta and Mehdia had the lowest infection rates (12.5 and 11.1%, respectively).

Although grapevine-infecting viruses of the new introduced varieties in Tunisia have been already studied, the present work is the first one focused on native varieties. It was conducted on the gemplasm collection of INRAT and provides some clear highlights on the sanitary status of the Tunisian autochthonous grapevines, and reveals the healthy status of the wild genotypes. The main result to point out is the relatively high infection rate (40.4%) of the tested cultivars, with the prevalence of the mealybug transmitted viruses GLRaV-1 and GLRaV-3.The status of Tunisian autochthonous grapevines may be considered better than that of the introduced varieties (9, 11) than those of native grapevines from Spain (3) and Croatia (6), but similar to that of the Serbian ones (17). The high incidence of GLRaV-3 is in line with previous studies on introduced varieties in Tunisia (9, 11), in the Mediterranean and Near Eastern countries (4) and in the rest of world (7, 15, 20). The large presence of the mealybug *Planococcus ficus* and its implication in the transmission of GLRaV-3 (8, 10) could in part explain the widespread of this virus in Tunisia. Compared to each other, southern regions showed a better sanitary status (19.7% infection) than that of northern regions (61.4% infection). This could be due to the large presence of mealybug vectors in the north, in addition to the presence of infected introduced varieties which represent a source of infection. In the south, the infection rate decreased to 0% in Kerkenah cultivars, while in the north it reached 100% in Rafraf cultivars and near 80% in other regions like Zaghouan and Baddar. Notwithstanding the high virus infection levels detected in the autochthonous cultivars, some accessions possess the minimum sanitary requirements and constitute potential sources of propagating material for the establishment of new germplasm collection. As a conclusion, this study gives evidence that appropriate sanitation procedures will be necessary for many Tunisian native grapevine cultivars in order to provide the national certification program with healthy mother plants.

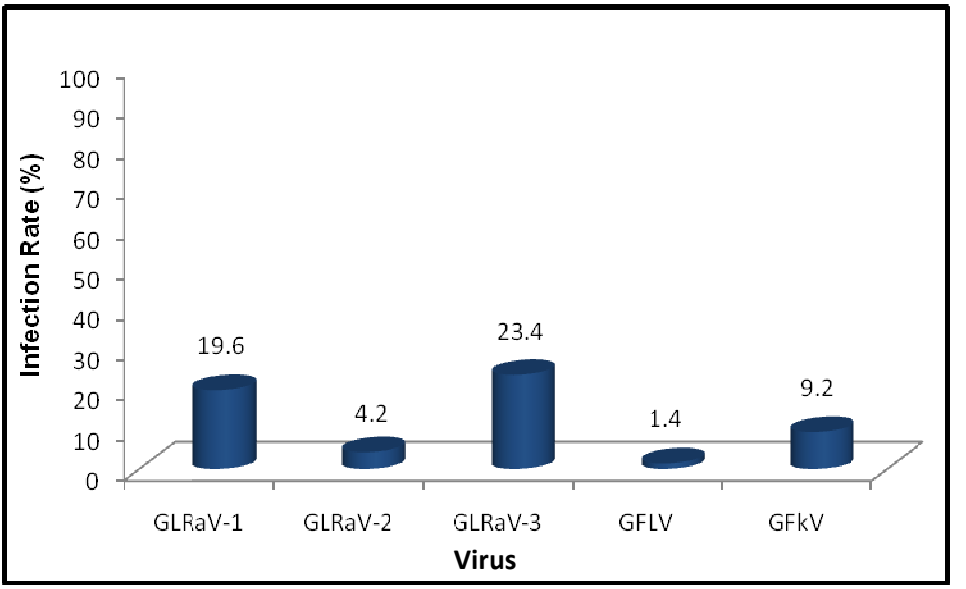

Fig. 1. Mean prevalence (%) of viruses infecting Tunisian autochthonous grapevine cultivars.

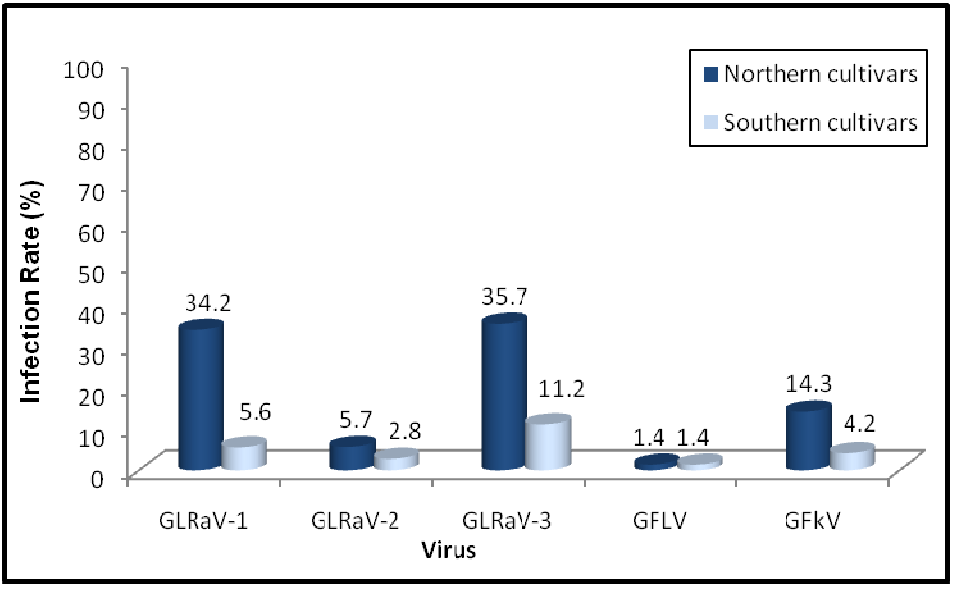

Fig. 2. Comparative incidence of viruses in grapevine cultivars collected from the northern and the southern regions of Tunisia.

Table 1. Virus incidence (%) of Tunisian grapevine cultivars in relation to their origin

Region	Number of tested cultivars	GLRaV-1	GLRaV-2	GLRaV-3	GFLV	GFkV	GLRaV-1 + GLRaV-3	Total infection
Northern regions								
RafRaf	18	55.5	0	77.7	5.5	33.3	38.8	100
Kef	8	0	12.5	0	0	0	0	12.5
Balta	10	10	0	20	0	10	0	40
Bargou	8	12.5	12.5	0	0	0	0	25
Baddar	4	0	0	25	0	75	0	75
Zaghouan	5	60	0	80	0	0	60	80
Djebba	14	50	14.3	21.4	0	0	14.3	64.3
Sbiba	3	66.6	0	33.3	0	0	33.3	66.6
Southern regions								
Mahdia	9	0	0	11.1	0	0	0	11.1
Sfax	8	12.5	12.5	12.5	0	12.5	12.5	25
Kerkena	9	0	0	0	0	0	0	0
Gabes	12	8.3	0	0	8.3	8.3	0	16.6
Djerba	8	0	12.5	37.5	0	0	0	37.5
Tozeur	12	8.3	0	8.3	0	8.3	0	25
Degueche	5	20	0	20	0	0	0	20
Nafta	8	0	0	12.5	0	0	0	12.5

RESUME

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L'incidence des infections virales a été étudiée dans la collection de vignes autochtones de l'Institut National de la Recherche Agronomique de Tunisie. Dans cette collection, 162 différents cultivars autochtones ont été maintenus, y compris de nombreux écotypes spontanés venant de différentes régions viticoles tunisiennes. Toutes les accessions ont été échantillonnées et analysées par DAS-ELISA pour la présence des virus 1, 2 et 3 associés à la maladie de l'enroulement foliaire de la vigne (GLRaV-1, -2, -3), le virus du court-noué de la vigne (GFLV), le virus de la marbrure de la vigne (GFkV) et le virus de la mosaïque de l'arabette (ArMV), en utilisant des antisérums polyclonaux. Presque tous les principaux virus infectant la vigne, à l'exception de l'ArMV, ont été détectés dans les cultivars testés. A l'inverse, toutes les accessions de vignes spontanées se sont montrées exemptes des mêmes virus. Parmi 141 cultivars soumis au test DAS-ELISA, 40,4% étaient infectés par au moins un virus. GLRaV-3 était le virus le plus dominant (23,4%), suivi par GLRaV-1 (19,6%), GFkV (9,2%), GLRaV-2 (4,2%) et GFLV (1,4%). Les cultivars provenant des régions du nord (61,4%) étaient plus infectés que leurs homologues des régions du sud (19,7%).

Mots clés: DAS-ELISA, incidence, Tunisie, vignes autochtones, virus

ملخص

محفوظي نعيمة ومنيرة حربي-بن سليمانومنال العيروإلهام السالمي وحُميْدة بن حمدة. انتشار الفيروسات التي تصيب أصناف العنب المحلية في تونس. Tunisian Journal of Plant Protection 9: 111-118.

أجريت هذه الدراسة الخاصة بالإصابات الفيروسية على مجموعة من أصناف العنب المحلية الموجودة بالمعهد الوطني للبحوث الزراعية بتونس. تحتوي هذه المجموعة على 162 صنفا مختلفا متأتية من عدة جهات تونسية، بما في ذلك العديد من الطرز البرية. أخذت عينات من كامل الأصناف ووقع تحليلها سيرولوجيا بواسطة أمصال متعددة التنسيل-DAS (ELISA) للبحث عن تواجد 6 فيروسات هي- Grapevine leafroll associated viruses 1, 2, 3 (GLRaV-1, -2, -3) و Grapevine fanleaf virus (GFLV) و Grapevine fleck virus (GFkV) و Arabis mosaic virus (ArMV). أظهرت التحاليل السيرولوجية أن كل الفيروسات التي وقع البحث عنها متواجدة ما عدا ArMV، وأن جميع الأصناف البرية خالية تماما من هاته الفيروسات. من بين 141 صنف مزروع تمت مراقبتها، هناك 40.4%مصابة بأكثر من فيروس. أما توزيع الفيروسات حسب نسبة الإصابة فهي كالتالي: GLRaV-3 (23,4%)وGLRaV-1 (19,6%)وGFkV (9,2%)وGLRaV-2 (4,2%)وGFLV(1,4%). وأظهرت الدراسة أخيرا أن الأصناف المتأتية من الجهات الشمالية أكثر إصابة(61,4%)من مثيلاتها من الجهات الجنوبية(19,7%).

كلمات مفتاحية: تأثير، تحليل سيرولوجي، تونس، عنب محلي، فيروسات، DAS-ELISA

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