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Survey of Grapevine Viruses in Jordan

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ABSTRACT

Field surveys were carried out in grapevine growing areas during the growing season 2003-2004 to evaluate the phytosanitary status of grapevines in Jordan. Samples from 1025 grapevines were collected and individually tested by Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA). Results showed that viruses were widespread in all orchards surveyed. About 33.6% of the samples (344 out of 1025) were virus-infected by one (25.1%), two (6.1%) or more (2.3%) viruses. However, remarkable differences in incidence were observed between the different viruses. The most prevalent being the Grapevine Leaf Roll associated Virus 1 (GLRaV-1) (13.7%) followed by Grapevine virus A (GVA) (12.2%), Grapevine Leaf Roll associated Virus 3 (GLRaV-3) (6.6%), Grapevine Fan Leaf Virus (GFLV) (5.0%), Tomato Ring Spot Virus (ToRSV) (4.7%), and the lowest was Grapevine Leaf Roll associated Virus 2 (GLRaV-2) (2.4%). Infection percentages were higher in nurseries (51.7%) than in commercial vineyards (30.6%).

Keywords: Surveys, GVA, GLRaV-1, 2, and 3, ToRSV, GFLV, DAS-ELISA.

1. INTRODUCTION

Grapevine (*Vitis vinifera*) is the second major crop after olives in Jordan. The area planted to grapevines constituted about 4.4% of the total fruit area in 2003 (Anonymous, 2003). A significant introduction of new grapevine varieties from a broad, mainly seedless varieties has taken place in these last years, giving a strong impulse to the renewal of Jordanian varietal platform. Sustained efforts to ensure high, reliable yields of grapevine are continuously threatened with damage to production caused by numerous diseases. Virus-caused diseases rank as the most economically damaging of any grapevine diseases because, in contrast to most fungal and bacterial diseases, once infected, the canes remain systemically infected for life with no respect for a cure (Sutic *et al*., 1999). Forty-four different viruses belonging to five families and sixteen genera have been identified in grapevines (Hadidi *et al*., 1998).

Virus seriously disrupts the structure and all functions of infected grapevine plants. Damaging effect of viral infections is expressed by various types of symptoms. First, and most important, they reduce grape yield and quality, often also reducing the productive life of grapevine canes. In production of grapevine stock, some viruses prevent rootstock and scion unions (Martelli 1993) (i.e., the cause of incompatibility). The extent damage depends on the characteristic of individual viruses and their strains, the susceptibility of a grapevine cv., the mode of virus transmission and the presence of the vector (Martelli, 1993; Sutic *et al*., 1999 and Andert-Link *et al*., 2004).

The phytosanitary status of grapevines with respect to virus diseases is little known in Jordan, the only available information stemming from two surveys conducted in recent years (Bosci *et al*., 1995; Al-Tamimi *et al*., 1998). Therefore, this study was conducted to provide information about the overall prevalence of grape viruses on the national level. Tests were conducted to determine the incidence of two viruses belonging to the nepovirus group, GFLV and ToRSV, three viruses that are commonly associated with grapevine leafroll disease i.e. GLRaV-1, 2 and 3 and one virus associated with rugose wood complex, (GVA).

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According to Martelli (1993), cortical tissues are excellent antigen source for nepoviruses and phloem-limited viruses (closteroviruses). On the other hand, for detection of nepoviruses (GFLV and ToRSV) antigen concentration is usually highest in new flushes of growth, but for closteroviruses (LRaV1-7) aged leaves represent a better antigen source than young leaves.

2. MATERIALS AND METHODS

Field Survey and Sampling

Field inspections were carried out during spring of 2003-2004 in the grapevines growing areas (Irbid, Ajlun, Jarash, Mafraq, Zarqa, Amman, Balqa, Jordan Valley, Madaba, Ramtha, Rum and Ash shawbak) of Jordan. Samples were collected from 57 commercial orchards and 10 nurseries throughout the traditional areas of cultivation.

A total of 1025 cane or leaf samples were collected randomlly from symptomatic and symptomless grapevines. During each survey, representative disease samples were tested in the greenhouse on a selected range of indicator plants.

Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA)

DAS-ELISA tests were applied for detecting the main viruses that cause problems and economic losses worldwide, GVA, GFLV, GLRaV-1, 2 and 3 and ToRSV.

Leaves or canes scraping were homogenized in extraction buffer (Tris-buffer pH 8.2, containing 0.8% (w/v) NaCl, 0.05% (v/v) Tween-20, 1% polyethylene glycol (PEG) MW 6,000, and 2% PolyVinylPyrrolidone (PVP) MW 20,000 at 1:10 (w/v) dilution. ELISA was carried out in polystyrene microtitre plates, following Clark and Adams (1977). Commercial kits (Bioreba AG, Reinach, Switzerland) were used with duplicate wells per sample for each of the viruses tested. Positive and negative controls were also included in each test. The absorbance at 405 nm of each well was measured using Expert Plus ELISA reader. Results were judged to be positive if the mean absorbance (405 nm) was greater than twice those of the negative control.

Biological Assay

Samples from the selected vines were used to inoculate a range of herbaceous hosts for the presence of mechanically transmissible viruses. Leaves were ground in mortar in the presence of 0.01 M phosphate buffer pH 7.8, containing 0.001 M sodium-Diethyldithiocarbamate (Na-DIECA), 2.5% nicotine and activated charcoal (100 mg:ml, w/v), and were rubbed on carborundum dusted leaves of *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Nicotiana benthamina*, *N. clevelendii*, *N. glutinosa*, *Phaseolus vulgaris* and *Vigna unguiculata*. The inoculated plants were kept under green house conditions and checked for symptoms expression.

3. RESULTS

Field Survey and Sampling

Depending on symptoms and due to period of the survey, the only symptoms observed and identified with reasonable confidence in the field were those typical of GFLV, ToRSV and GVA. Other symptoms, resembling those induced by leafroll were not observed. Fanleaf symptoms were best seen early in the growing season. Young leaves were asymmetric, deformed, showing excessive dentition (abnormally sawtoothed leaf edge) and gathering together of the main veins at the base of the leaf blade to resemble a lady's fan. Some leaves showed extensive patches of bright yellow, diffusing into a yellow-green pattern. ToRSV was recovered from plants that showed shortened internodes with small distorted leaves and yellow vein resemble those described for fanleaf. GVA was consistently associated with swelling above the bud union and a marked difference between the diameter of scion and rootstock.

Some of grapevines showed virus-like symptoms, systemic green or yellow mosaic, ring and line patterns, flecks and leaf malformation, but these symptoms were not associated with any of the tested viruses.

Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA)

DAS-ELISA tests showed that about 33.6% of the samples (344 out of 1025) were virus infected by one (25.1%), two (6.1%) or more (2.3%) viruses. Infection percentage were higher in nurseries (51.7%) than in commercial vineyards (30.6%).

Grapevine viruses were widespread in all regions surveyed. The infection percentage ranged from 8.4% to 57.1%. However, remarkable differences in incidence were observed between the different regions, the most affected being Amman (57.1%) followed by Rum

(43.8%), Mafraq (42.3%), Jarash (23.4%), Madaba (37.5%), Jordan Valley (37.1%), Balqa (15.3%), Ramtha (30%), Ajlun (27%), Zarqa (18.9%), Ash shawbak (15.4%) and the lowest was Irbid (8.4%) (Table 1).

GLRaV-1 was the most widespread virus (13.7%). The virus incidence ranged from 2.8 in Balqa orchards to 39.2% in Balqa nurseries. The highest percentage of infection was recorded in nurseries (33.1%), while the rate of infection in commercial orchards was 10.5% (Table 1).

GVA was the second most frequent virus after GLRaV-1, as mean percentage of infection reached 12.2%. GLRaV-3, GFLV, and ToRSV were also detected to a lesser extent; their incidence being 6.6, 5.0, and 4.7%, respectively. GLRaV-2 existed with a relatively low percentage being 2.4%.

Single GLRaV-1 infections were detected in 25.6%, GVA in 18.3%, GLRaV-3 in 13.7%, ToRSV in 9.6%, GLRaV-2 in 3.8% and GFLV in 3.8% (data not shown). Multiple infections of these viruses were also found. GVA with GFLV was present in 3.8%, GVA plus GLRaV-1 in 4.7%, GVA with GLRaV-2 in 0.3%, GVA with GLRaV-3 in 1.5%, GVA with ToRSV in 1.2%, GFLV with GLRaV-1 in 2.7%, GFLV with GLRaV-2 in 0.3%, GFLV with GLRaV-3 in 0.3%, GLRaV-1 plus GLRaV-2 in 0.3%, GLRaV-1 plus GLRaV-3 in 0.3%, GLRaV-1 plus ToRSV in 1.5%, GLRaV-2 with GLRaV-3 in 0.6%, GLRaV-3 with ToRSV in 1.2%, and finally, the combined presence of three or four viruses was detected in 7.0% of the infected grapevine sources (Table 2).

All mixed infections showed 20 virus combinations. The association of GVA and GLRaV-1, apart from the combination observed, proved to be the most widespread one (41.3%) (Table 2).

Biological Assay

Based on biological assay, few isolates were recovered from leaves collected from naturally growing grapevine in Jordan. One isolate was selected to represent other isolates based on its reaction with *C. amaranticolor* and *C. quinoa*. The representative isolate was identified as ToRSV. In all symptomatic plants the presence of the ToRSV was ascertained by DAS-ELISA. The inoculated leaves of *C. amaranticolor* and *C. quinoa* developed small chlorotic local lesions followed by systemic symptoms. However, no symptoms were observed on plants inoculated with extract from GVA, GFLV, GLRaV-1, 2 and 3, Symptomless assay plants were negatively reacted with ELISA test.

4. DISCUSSION

The overall presentation of symptoms in virus affected vines with other overlaying and complicating infections can be expected to be confusing. The symptoms of any one disease caused by a specific virus may be varied by the presence of another virus, not only in their intensity, but also in their appearance. Another point of interest to note is that it is also known that climatic factors, such as cold dry springs, can induce or significantly exacerbate symptoms seen in viruses affected vines.

This survey reveals the occurrence of GLRaV-1, 2 and 3, GFLV, and GVA on grapevines and reports the occurrence of ToRSV in Jordan for the first time. The high level of infection percentages points to the use of diseased material as a possible primary cause of sanitary deterioration. The occurrence of these viruses in combination with each other viruses and virus-like pathogens is known to give rise to more serious conditions that may present with confusing symptoms. These more serious conditions are very possibly impacting significantly on our viticultural production. Compared with the incidence of virus diseases on grapevine reported by Al-Tamimi *et al*. (1998), the percentage of positive samples detected in this study was lower (33.6% vs. 60%). This difference may be due the time of sampling. Samples for this survey were collected from March until the end of July; those of Al-Tamimi *et al*. (1998) during late autumn. However, the influence of tissue type and seasonal influence on virus detection of some grapevine viruses were examined by Rowhani e*t al*. (1992).

Laboratory testing by ELISA is proved to be a valuable tool for investigating vineyards problems because field diagnosis of grapevine virus diseases can be difficult (Martelli, 1993). Symptoms displayed in the field are rarely unique to a particular disease. In addition, some infected vines may not show any symptoms indicative of their disease status. More often, with most grapevine virus diseases, diagnostic symptoms only occur during certain times of the year. Leafroll, for example, causes leaves to redden in red-fruited varieties in the late summer and fall. Examination of these vines in the spring would give no indication of their disease status.

ELISA tests showed that GLRaV-1 and GVA

infections of grapevine are widespread throughout vineyards of Jordan. GLRaV-1 was the most frequent of the single virus infections. This virus combined with GVA was also the most prevalent of the mixed infections. These findings agreed from those reported for North Italy (Credi and Giunchedi, 1996).

The relatively low incidence of ToRSV and GFLV in grapevine suggests that transmission by nematode is not an efficient way of spreading nepoviruses under field conditions.

In terms of virus infection level, the overall situation of grapevine viral diseases in Jordan, seems slightly better than neighboring countries i.e. Lebanon (53%) and Egypt (78%), where comparable surveys were recently conducted (Haidar *et al*., 1996; Ahmed *et al*., 2003).

The detection of the GVA, GFLV, GLRaV-1, 2 and 3, and ToRSV reported here are the prelude to a more extensive diagnostic study on virus and virus-like diseases of grapevines in Jordan. The results are significant and will substantially influence the direction of grapevine import policy. While it is beyond the scope of this study to propose specific changes to plant-protection import policies.

Since grapevine virus diseases are spread with infected rootstocks, buds, cuttings and rootings, it is important to use certified virus-free planting, grafting or budding stock in order to keep the spread of virus diseases under control. At a national level we need to begin in a concerted process of removing these viruses from our lines of germplsam stocks, and to introduce methods of screening for them in propagation material and nursery stocks.

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حصر فيروسات العنب في الأردن

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ملخـص

تم إجراء مسح حقلي في مناطق زراعة العنب في الأردن خلال موسم النمو (2003-2004) لتقصي الحالة الصحية للعنب في الأردن. جمعت 1025 عينة وفحصت باستخدام تقنية الإليزا (DAS-ELISA). أشارت النتائج إلى انتشار الفيروسات في جميع البساتين التي تم مسحها. بلغت نسبة إصابة العينات 33.6 % (344 من 1025)، منها: 25.1% إصابة بفيروس واحد، 6.1% إصابة بفيروسين، و2.3% إصابة بأكثر من فيروسين. ولوحظ اختلاف بين نسبة الإصابة بالفيروسات المختلفة حيث بلغت النسبة المئوية للإصابة بفيروس التفاف الورقة رقم 1 (GLRaV-1) أعلى نسبة اصابة 13.7%، تلاه فيروس ينقر خشب ساق الكرمة A (GVA) بنسبة 12.2%، وفيروس التفاف الورقة رقم 3 (GLRaV-3) بنسبة 6.6%، وفيروس الورقة المروحية (GFLV) بنسبة 5%، وفيروس التبقع الحلقي للبندورة (ToRSV) بنسبة 4.7%، وكان فيروس التفاف الورقة رقم 2 (GLRaV-2) الأقل انتشارا حيث بلغت نسبة الاصابة به 2.4%. وقد سجل الانتشار الأعلى للإصابات في المشاتل (51.7%) مقارنة مع البساتين التجارية (30.6%).

الكلمـات الدالـة: مسوحات، فيروس ينقر خشب ساق الكرمةA، فيروس التفاف الورقة رقم 1، 2، 3، فيروس التبقع الحلقي للبندورة، فيروس الورقة المروحية، فحص الإليزا.

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