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*Original article*

# First Report of Barley Stripe Mosaic Virus Infecting Barley (*Hordem Vulgare* L.) In Al-Jabal Al-Akhdar- Libya

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**ABSTRACT**

***Aims****. The study aimed to assess the presence of barley stripe virus isolated from the willow field in Al-Jabal Al-Akhdar for the first time in Libya.* ***Methods****. The barley stripe mosaic virus (BSMV) field tested and the virus was transferred by mechanical transfer technique. The virus was identified using cytological assays and the polymerase chain reaction (PCR).* ***Results****. A survey was conducted to detect infection with Barley mosaic virus (BSMV) in a field in Al-Safsaf, Al-Jabal Al-Akhdar, during the months of February and March of 2022. Symptoms associated with the virus were identified, as well as infection was confirmed using the polymerase chain reaction (PCR) technique. Tests showed the presence of the BSMV virus in the field where the Barley crop was surveyed. The virus was transmitted mechanically to the host, Hordeum vulgare vr. ACSAD1877, where the virus was multiplied. The characteristics of the BSMV isolate were studied, and thin sections of Barley leaves infected with the BSMV isolate were examined. Some changes were evident. The cellular activity associated with the infection, including the change in the shape of the chloroplasts and the appearance of cytoplasmic internal particles in which the virus particles are concentrated, while the nucleus was not affected by the infection or showed particles of the virus. BSMV isolate with a genetic content of RNA divided into parts with molecular weights of 3 and 2.5 kb for each of the alpha and beta genome parts. The alpha and beta genome parts were amplified separately using the polymerase chain reaction (PCR) technique, using specialized primers for each part of the parts. The genome and when ten different cutting enzymes were added to the alpha and beta parts of the genome, the different patterns of the cutting became clear, which reflect the difference in the nucleotide sequences involved in building the different parts, and this confirms that the parts are not similar and confirms that the virus has many genetic components.* ***Conclusion****. Our findings suggest the importance of identifying viruses that infect grassy crops of important economic importance in Libya, including barley and wheat, because these viruses are pathogenic pests that cause heavy losses to these crops.*

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## INTRODUCTION

Barley stripe mosaic virus (BSMV) was the first cereal virus to be recognized as causing disease problems. As early as 1910, a distinct disease syndrome was noticed in North America. Leaves collected by 1913, showed clear signs of a disease which was named “false stripes” by Ibra Conners in 1924. However, the viral etiology of the disease was firmly demonstrated by mechanical transmission only in 1951 [1] and the detection of rod-shaped particles in infected plants was confirmed subsequently by Gold *et al*., [2]. BSMV occurs world-wide, its occurance has been reported from: -Europe: Denmark [3]; Hungary [4]; Romania [5]; USSR [6]; Yugoslavia [7]; Germany [8; 9]. -Asia: China [10]; Israel [11]; Korea [12]; Lebanon and Pakistan [13]; Syria [14]; Turkey [15]. Africa: Egypt [16; 17]; Tunisia [18; 19]. -North America: Mexico [20]; USA in most regions but mainly in Montana and North Dakota [21], Brazil [22]; Peru [23], Oceania: Australia [24]; New Zealand [25].

BSMV is a linear, positive sense, single stranded RNA virus composed of three genome components designated α, β and γ in order of decreasing molecular weight [26]. The α and β genomes are 3.8 and 3.3 kb in size, respectively, but γ genome differs in size depending on the strain [27]. BSMV strains differ in the number of RNA components that can be resolved by gel electrophoresis [28]. The high molecular weight RNAs of the Type and Russian strains separate into two major components that are about 3800 and 3300 nucleotides (NT) respectively. The ND18 and Norwich strains have three components estimated to be 3800, 3300 and 2800 NT, while the Argentina Mild (AM) and North Dakota161 strains have four components with sizes estimated to be 3800, 3300, 2800 and 2600 NT [29]. This size variation initially led to some confusion about

the requisite number of RNA components necessary for infectivity [30]. Infectivity experiments with separated RNAs first suggested that the Norwich strain of BSMV has a tripartite genome [31]. Other experiments showed thatthe Type and ND18 strains were relatively stable, but that the AM strain couldbe subcloned into two- and three-component isolates similar to the Type andND18 strains [32].
BSMV causes a serious disease of Barley, wheat and oats and apparently has spread throughout the world by distribution of infected seed [33]. In some instances, losses caused by BSMV are so severe that farmers have ceased Barley production [34]. The extent of disease development is proportional to the degree of seed infestation since the virus is initially spread to adjacent plants by mechanical contact from foci originating from infected seeds. Although BSMV is pollen transmitted [35] and its spread from diseased to healthy plants occurs through leaf contact [36], there are no vectors capable of efficient soil or aerial transmission, so the disease can be controlled by eliminating virus from seed stocks [37,38].
As well as cytological alterations, BSMV may induce changes in the genomes of infected plants. In barley, an increased frequency of triploids, aneuploids and chromosomal breakage is observed in progeny of infected barley [39]. BSMV also is correlated with an increased mutation rate, designated "aberrant ratio", during infection of maize [40]. This phenomenon appears to be an indirect result of BSMV infection [41]. Infected barley leaves have less chlorophyll than non-infected leaves, as is obvious from the mosaic pattern [42]. Other changes include reduced amounts of chloroplast ribosomal RNA [43-44] and decreased rates of synthesis of chloroplast proteins during the same time that RNA-dependent RNA polymerase activity increases [45].
The aim of this work was to screen the Barley field for Barley Stripe Mosaic Virus (BSMV) in Al-Safsaf region in Jabal Al-Akhdar. Further isolation and molecular characterization of BSMV to identify the virus that infects the most important economic crops in Libya.

## METHODS

***Survey in Barley fields***

A survey of bearly stripe mosaic virus (BSMV) infection was conducted in one barley field in the Safsaf region during the months of February and March 2022. In the survey, one sample was collected from a barley field that showed symptoms suggestive of a viral infection and tests were carried out on it, which is a mechanical transfer test, and then tested using the Polymerase Chain Reaction (PCR) technique.

***Mechanical transmission and propagation***

BSMV was propagated in Hordeum vulgare vr. ACSAD1877" by mechanical inoculation from virus infected leaves. BSMV infected leaves were ground in mortar. The inoculation buffer (g/100ml 0.01Mphosphate buffer pH 7, 0.5g Na2SO3 and 1% carborand) was added to the infected tissue at a ratio of 1:10(tissue weight: inoculation buffer volume). Leaves were wounded during inoculation by carborandto aid viral entry [46].

***Cytopathological effects of BSMV infecting Barley leaves***

Systemically infected Hordeum vulgare vr. ACSAD1877 leaf tissues (1×3 mm) were fixed in formalin / glyceridedehyde (4:1), washed 3x in sodium phosphate buffer, pH 7.0, and post fixed in osmic acid for 1.5h. then further washed in phosphate buffer. After dehydration in an acetone series, infiltration was carried out in acetone/ Epon mixture (1:1) for 24 hr. The tissue was embedded in Epon mixture with hardener (DMP30) and stained with 1% toluidine blue. Semi-thin sections were cut for light microscopic examination. The sections for electron microscopy were further stained with uranyl acetate and lead citrate and examined by the use a JEOL-CX100 electron microscope [47].

***Extraction of the genomic RNA of (BSMV) isolate***

Total RNA was extracted from leaf samples using the Thermo scientific GeneJET Viral RNA purification kit (GE Healthcare, UK). 50µl of column preparation liquid was added to the center of spin column membrane, so that the membrane is entirely moistened. 200 µl of virus was loaded to an empty 1.5ml lysis tube, then 200µl of lysis solution (supplemented with Carrier RNA), and 50µl of proteinase K were added to the sample and mixed thoroughly by pipetting. The sample was incubated for 15min at 56˚C in a thermomixer, and centrifuged for 3-5 s at full speed to collect drops from the inside of the lid. 300 µl of ethanol (96-100%) was added to the sample, mixed by pipetting, incubated at room temperature for 3min, and centrifuged for 3-5 s at full speed to collect drops from the inside of the lid. The lysate was transferred to the prepared spin column preassembled within the wash tube and centrifuged 1min at 14,000 rpm. Flow through was discarded. The spin column was placed into a new 2ml wash tube.700µl of wash buffer1 supplemented with ethanol to the spin column was added and centrifuged for 1min at 14,000 rpm. Flow through was discarded. The spin column was placed into a new 2ml wash tube. 500µl of wash buffer 2 supplemented with ethanol to the spin column was added and centrifuged for 1min at 14,000 rpm. Flow through was discarded. The spin column was placed into a new 2ml wash tube. The spin column was centrifuged for 1min at 14,000rpm. Flow through was discarded. The spin column was placed into a new 1.5ml elution tube. 50µl of eluent preheated to 56˚C was added to the center of spin column membrane. The column was incubated for 2 min at room temperature and centrifuged for 1min at 14,000 rpm. Flow through was discarded. Total RNA extracted was stored at -20°C until use for PCR analysis.

***Synthesis of cDNA for the isolate (BSMV) RNA***

First-strand cDNA was synthesized using Moloney Murine Leukemia Virus reverse transcriptase(Fermentas,USA)and its buffer (5X)[50mM Tris-HCl(pH 8.3 at 25˚C), 250 mM KCl, 20 mM $MgCl_2$ and 50 mM DTT ] in presence of random hexamer primer (Promega,USA). 5µl of RNA was added to (10 µl (5x) RT-Buffer, 5 µl (25mM) dNTPs, 5 µl of primer, 0.5 µl (20u/ µl) of RT-enzyme, 24.5 µl $H_2O$). The mixture was incubated at 37˚C for 60minutes, then at 70˚C for 10minutes [48].

***Amplification of the α and β and genomic cDNAs of isolate (BSMV) by polymerase chain reaction (PCR)***

PCR reaction was performed in a total volume of 50 µl containing 5 µl (10x) Taq buffer (Fermentas, USA) (100mMTris-HCl pH 8.8 at 25˚C, 500mM KCl),1µl Taq polymerase(5u/µl) (1min/kb) (Fermentas, USA),3 µl $MgCl_2$ (25mM),4 µl (20mM) dNTP mixture,3 µl from each primer (20 Pmol/µl) ,2 µl cDNA and 29 µl $H_2O$. The reaction mixtures were subjected to amplification as follows: Denaturation of DNA template at 95˚C for 2 min, followed by 35 cycles of amplification with denaturation at 95˚C for 2min, annealing at 52˚C in α and β with extension at 72˚C for 10 min. The PCR system (Gene Amp 9700 thermocycler, Applied Biosystem (ABI), USA) was used for performing PCR amplification.

***Table 1: Specific synthetic oligonucleotides primers used for cloning (BSMV) isolate genes.***

| Primer Name | sequence (5'-3') | Expected product size (bp) | Refe. |
|---|---|---|---|
| a5Pst1 | 5'-CCCTGCAGGTATGTAAGTTGCCTTTGGGTGTAAAATTTCTTGC-3' | 3011 kbp | **Gustafson, G. et al. (1989).** |
| BSMV3Xba | 5'CCTCTAGATGGTCTTCCCTTGGGGGACCGAAGCTGAGCTTCGGC3' | | |
| b5Sma1 | 5'-CCCCCGGGGTAAAAGAAAAGGAACAACCCT-3' | 2500kbp | **Gustafson, G. and Armour, S. L. (1986).** |
| BSMV3BamH1 | 5'CCGGATCCTGGTCTTCCCTTGGGGGACC-3' | | |

***Table 2. Thermal cycling conditions used for PCR protocol for (BSMV).***

| Final extension | Elongation | Annealing | Denaturation | Cycles |
|---|---|---|---|---|
| 72 ˚C /10 min | 72 ˚C /2min | 52 ˚C /2min | 95 ˚C /2min | **(35)** |

## RESULTS

***Survey of AL-Safsaf Barley fields***

A Barley field in Al-Safsaf area of Al-Jabal Al-Akhdar was surveyed for symptoms of (BSMV) diagnosis in February and March 2022. Barley plants showing characteristic symptoms suspected to be associated with BSMV were detected. Leaves of infected plants were yellow with striped mosaic symptoms of light green, yellow or white color as shown in Figure (1a-b). Then one sample was taken and placed in a plastic bag and sent to the Delta Laboratory for Scientific Services in Egypt to conduct tests.

**(a)** **(b)**

***Fig. 1a-b. Diagnostic symptoms of BSMV infection in a Barley field surveyed in willow Barley in 2022-growing season. The leaves of the plant appear chorionic due to the mosaic symptoms of striped light green, yellow or white***

***Mechanical transmission and propagation***

One isolate of (BSMV) isolated from willow Barley plants was used. (BSMV) was propagated in Barley cultivar Hordeum vulgare vr. ACSAD1877 by mechanical inoculation up to 10 days after inoculation and until symptoms of (BSMV) appeared. This procedure was repeated several times until the virus spreads in sufficient quantities in the host tissue.

***Fig. 2. Symptoms of (BSMV) on mechanically inoculated Hordeum vulgare vr. ACSAD1877" leaves. Leaves were chlorotic due to linear light green, yellow or white striping mosaic symptoms.***

***Electron microscopy of thin sections of (BSMV)-infected Barley***

Cytopathological effects resulting from infection by (BSMV) of Hordeum vulgare vr. ACSAD1877 were detected by electron microscopy of thin sections of Barley leaves as shown in Fig. (3). Thin section of healthy, uninoculated leaves Fig. (3a) were compared to those of BSMV inoculated leaves 15dpi (Fig.3b-f). In BSMV infected leaves, the morphology and shape of chloroplasts of infected Barley cells were abnormally rounded or distorted Fig. (3b). Chloroplasts contained globular or balloon-like cytoplasmic inclusions surrounding the chloroplast which maintained an ovoid shape Fig. (3b&c). The endoplasmic reticulum was also dilated Fig. (3d), numerous small vesicles appeared between the inner and outer membrane of the chloroplast envelope together with disorganized grana Fig. (3c). Tubular rod-shaped virus-like particles were found within the cytoplasmic inclusions, in the cytoplasm surrounding the chloroplast Fig. (3b&e). No cytopathological effect was detected on the nuclei of infected cells. It seems that nuclei contained a much lower concentration of virus particles or none, in contrast to the cytoplasm Fig. (3f). No detectable alterations of the cell wall were visible. Enlargement in the size of the cell vacuole was also detected in infected Barley leaves Fig. (3f).

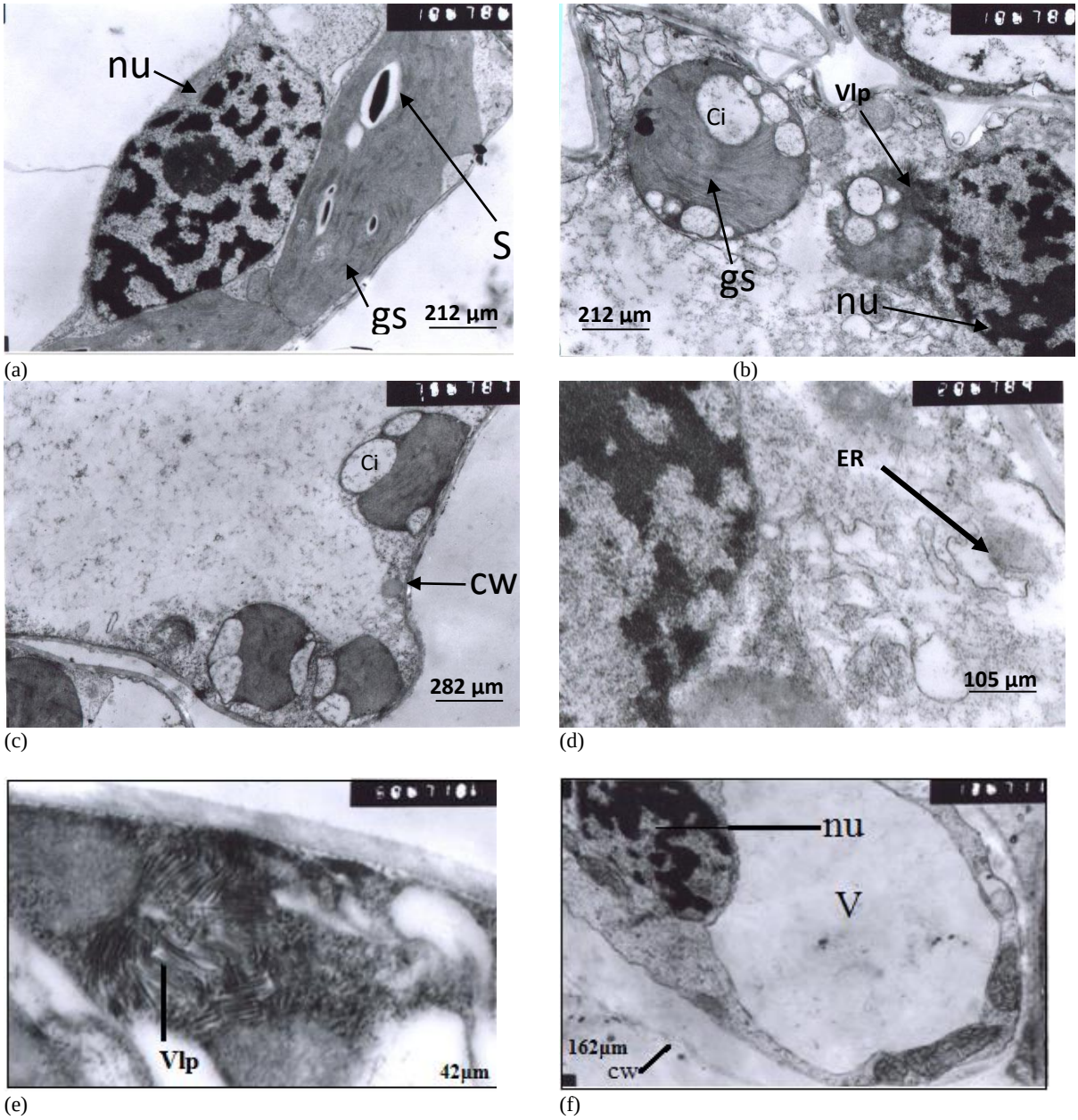


***Fig. 3. Transmission electron microscope images of thin sections of Hordeumvulgarecr. ASCADA 1877 leaves infected with (BSMV). Thin section of healthy, uninoculated leaves (a), (BSMV) inoculated leaves 15 dpi (b-f). S =starch granules; Ci = cytoplasmic inclusion; Vlp = virus like particles; nu = nucleus; cw = cell wall; gs =grana stacks; V= vacuole; ER= endoplasmic reticulum***

***Amplification of the α and β and genomic cDNAs of (BSMV)***

The synthesized cDNA was subsequently used with specific synthetic oligonucleotides designated for each of the genomic RNAs α and β to act as primers for the production of the corresponding α and β DNAs. PCR products were purified from the PCR reaction mixture and separated by

electrophoresis on 1% agarose gel in 1x TBE buffer. Results shown in Fig. (4) indicated the presence of PCR-amplified fragments of molecular weight 3 and 2.5 kb representing the synthesized α and β DNAs.

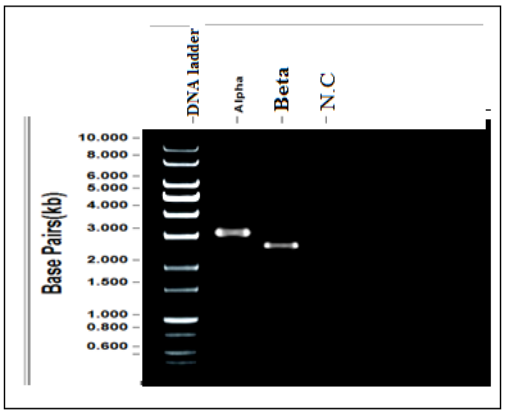


***Fig. 4. Electrophoretic mobility of (BSMV) isolate purified PCR products amplified from cDNA using specific synthetic oligonucleotides primers. Approximately 4 µl of (BSMV) isolate DNA was run through 1% agarose gel in 1xTBE buffer, stained with ethidium bromide and photographed. Lane M: Molecular weight standard 1 Kb plus DNA ladder (GIBCO BRL); lane1: alpha DNA; lane 2: Beta DNA.***

## DISCUSSION

Barley is one of the fodder grain crops of an economic nature in the world because it contains fiber in large proportions. It is a crop of great importance in Libya because of its direct connection to the livestock sector. Barley tolerates adverse environmental conditions, drought and irrigated water. The Barley crop is grown in the eastern region of Libya under Rainfed agricultural conditions.It is also grown in agricultural projects, where productivity reached 5 tons / hectare [49,50], where the total area cultivated with wheat and Barley crops in the year 1998/1999 was about 146,000 and 270,000 hectares, respectively [51]. The amount of Barley intended for human consumption was 160,000 tons.The annual per capita share is about 30.5 kg, and the self-sufficiency rate reached 100%, while the quantities of wheat intended for consumption amounted to 700,000 tons, and the annual per capita share reached 128.7 kg, and the self-sufficiency rate reached 10.8% [52,53].

Barley band mosaic virus was detected in a Barley field in Safsaf district, Libya, during the 2022 growing season. (BSMV) infection was detected by visual examination and molecular diagnosis. The symptoms appeared after mechanical transmission on the Barley plant Hordeum vulgare vr.1877 ACSAD 10 days after injection, the characteristic symptoms of the virus ranged from very light streaks to long streaks covering the entire leaf surface that can lead to fatal necrosis. BSMV destroys host plants by disrupting their chloroplasts, resulting in bright green, yellow or white striped leaves. Chloroplast loss reduces photosynthetic activity and thus reduces seed production [54]. Evidence of its occurrence has been reported in Africa, mainly in Egypt and Tunisia [55]. Prevalence of (BSMV) in the host Barley plant, Hordeum vulgare vr. Exada 1877 No research has been done on BSMV infecting Barley in Libya before, while four virus species have been shown to infect Barley plants, namely, Barley Yellow Dwarf PAV (BYDV-PAV) and Spotted Mosaic Virus (MSV) in Libya (Hatem Abu Akar , Safaa Ghassan Qamari, Fawzi Beshaya [56].

The cytoplasm of infected tissue was reported to contain large inclusion bodies with disordered masses of virions [57]. The alteration during infection in the shape and morphology of chloroplast were also reported by McMullen *et al*., [58]. As well as the disorganization of the grana. The absence of structural effects on the nuclei of BSMV infected cells, in comparison to uninfected cells, has also been reported and was attributed to the replication of (BSMV) in the cytoplasm [59,60]. Such suggestions have been confirmed by the ability to detect dsRNA which could be the replicative form of the virus by antisera against dsRNA in host cytoplasm, Antibodies were used to detect dsRNA in protoplastids of (BSMV) infected wheat root tips [61] and authors concluded that proplastids play a role in (BSMV) replication. Similar observations were seen for turnip yellow mosaic virus [62].

To prime the cDNA synthesis of BSMV isolate α and ß genomic RNA components, specific synthetic oligonucleotides were used. Following reverse transcription into first strand cDNA and by the use of the specific primers, Polymerase chain reaction (PCR) was used to amplify each of components. The electrophoretic mobility of the isolate α and ß and BSMV genomic components showed molecular weight estimates of 3 and 2.5 Kb. These estimates are in reasonable agreement with the previously mentioned electrophoretic estimates [63-64].

## CONCLUSION

The current study is the first record of virus (BSMV) on the barley crop; In Libya, based on the results Cytological and molecular. The exact definition of identity Viruses that affect these crops are essential for breeding plants with the aim of selecting and adopting cultivars resistant to viral infections Effective crop management is needed to prevent this.

***Conflict of Interest***

There are no financial, personal, or professional conflicts of interest to declare.

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# أول تقرير عن إصابة فيروس موزايك الشعيرالمخطط للشعير (*Hordeum vulgare L*) في الجبل الأخضر- ليبيا

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**المستخلص**

**الاهداف**. أظهرت الدراسة وجود فيروس شريط الشعير المعزول من حقل الصفصاف في الجبل الأخضر لأول مرة في ليبيا والذي تم التعرف عليه باستخدام النقل الميكانيكي والاختبارات الخلوية وتفاعل البوليميراز المتسلسل.(PCR) . **طُرق الدراسة**. تم إجراء مسح للكشف عن الإصابة بفيروس موزاييك الشعير (BSMV) في حقل في الصفصاف والجبل الأخضر وتم نقل الفيروس بطريقة النقل الميكانيكي .تم التعرف على الفيروس باستخدام المقايسات الخلوية وتفاعل البلمرة المتسلسل(PCR) . **النتائج.** تم إجراء عملية مسح للكشف عن الأصابة بفيروس التبرقش المخطط في الشعير (BSMV) في حقل بمنطقة الصفصاف بالجبل الأخضر وذلك خلال شهري فبراير ومارس من عام 2022. تم التعرف علي الأعراض المصاحبة للفيروس وكذلك التأكد من الإصابة باستخدام تقنية تفاعل البلمرة المتسلسل (PCR). أظهرت الاختبارات وجود الفيروس BSMV فى الحقل الذى أجري فيها المسح لمحصول الشعير تم نقل الفيروس بالطرق الميكانيكية إلي العائل Hordeum vulgare vr. ACSAD1877 " حيث تم إكثار الفيروس, حيث درست خصائص العزلة BSMV, وفحصت قطاعات رقيقة من أوراق الشعيرالمصابة بعزلة BSMV اتضح وجود بعض التغيرات الخلوية المصاحبة للإصابة ومنها التغير في شكل البلاستيدات الخضراء و ظهور جسيمات داخلية سيتوبلازمية تتمركز بها جزيئات الفيروس في حين أن النواة لم تتأثر بالإصابة أو يظهر بها جزيئات للفيروس. عزلة BSMV ذات محتوى جينى من ال RNAمقسم علي أجزاء بأوزان جزيئية 3و2.8 كيلو قاعدة لكل من أجزاء الجينوم الفا , بيتا وتم تضخيم أجزاء الجينوم الفا , بيتا كل علي حدة باستخدام تقنية تفاعل البلمرة المتسلسل (PCR) وذلك باستخدام بادئات متخصصة لكل جزء من أجزاء الجينوم وعند إضافة عشر إنزيمات قطع مختلفة علي أجزاء الجينوم الفا و بيتا اتضحت الأنماط المختلفة للقطع والتي تعكس الاختلاف في تتابعات النيوكليوتيدات الداخلة في بناء الأجزاء المختلفة وذلك يؤكد أن الأجزاء غير متشابهة وتؤكد أن الفيروس عديد المكونات الجينية. **الخلاصة** . تقترح النتائج التى توصلنا إليها أهمية تعريف الفيروسات التى تصيب المحاصيل النجيلية ذات الاهمية الاقتصادية فى ليبيا بما فى ذلك الشعير والقمح لأن هذه الفيروسات من الافات الممرضة التى تسبب خسائر فادحة.

**الكلمات المفتاحية**. الشعير - تفاعل البلمرة المتسلسل (PCR)- فيروس موزايك شريط الشعير.