Differentiation between Tomato Fusarium Wilt Isolates by ISSR Markers and Virulence Analysis

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ABSTRACT: Fusarium oxysporum. f. sp. lycopersici (FOT) causes tomato fusarium wilt, one of the critical fungal diseases causing severe losses in tomato production in Egypt. Eight isolates of F. oxysporum. f. sp. lycopersici were isolated from infected tomato plants collected from six Egyptian governorates. FOT4, FOT2, and FOT5 isolates were highly virulent, while FOT6 and FOT8 were moderately virulent, and FOT7, FOT3, and FOT1 were weakly virulent. All tested ISSRs primers were polymorphic and produced 81 DNA loci of various molecular sizes. DNA fragment patterns analysis showed 37 monomorphic and 44 polymorphic bands, with 54.32% polymorphism between the tested isolates. Among the tested isolates, primer UMC-840 had the highest level of polymorphism (85.71%), while primer HB-13 had the lowest level (33.33%). Dendrogram and ISSR band patterns were analyzed using the UPGMA method to detect the relationship between the eight isolates. The highest genetic homology (82.9%) was observed between FOT4 and FOT3 isolates, while the lowest genetic homology (63.5%) was observed between FOT1 and FOT2 isolates. The eight isolates were classified into three main clusters (A, B, and C). Cluster A includes two sub-clusters; sub-cluster A1 is divided into two groups, group1 consists of two subgroups, subgroup1 include FOT6 isolate, and subgroup 2 includes FOT4, FOT3, and FOT5 isolates (FOT4 and FOT5 were high virulent isolates), group2 has FOT8 isolate. Sub-cluster A2 includes FOT6 isolate. However, cluster B includes the FOT1 isolate, and cluster C includes the FOT2 isolate.

Keywords: Fusarium, isolates, polymorphism, dendrogram, and ISSR

INTRODUCTION

Fusarium species cause various diseases in many host plants (Summerell et al., 2003; Booth, 1971). These soilborne fungi are economically crucial because many of their members cause root rot and vascular wilt diseases in cultivated crops worldwide (Woo et al., 1998). Because of its global distribution in soil, F. oxysporum has been dubbed the global mycoflora (Parkinson, 1981). Fusarium species are commonly identified using their macro and microscopic characteristics. However, these characteristics are generally reported to be unstable (Szecsi and Dobrovolsky, 1983; Nelson et al., 1985). The morphological, biochemical, and allozyme characteristics are the most commonly used methods for pathogens identification, which require professional experience and are still subject to error (Kheterpal, 2006). Currently, pathogens identification is based on nucleotide sequence and genetic markers analysis. DNA markers used in genetic variation analysis are characterized by high genetic divergence and the ability to detect multilocus data in the genome (Anne, 2006). Molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Cleaved Amplified Polymorphic Sequences (CAPs), and Inter Simple Sequence Repeats (ISSR) are widely used in constructing fungi genetic maps. ISSR markers amplify microsatellite DNA sequences, which are highly varied and widespread in the genome. ISSR is an efficient multilocus technique that enables scoring multiple polymorphic loci and does not need any previous sequence information; however, it is limited by repeatability issues. Furthermore, the ISSR technique has high stability, accuracy, and efficiency compared to morphological assessment, so it has been widely used in genetic diversity studies (Williams et al., 1990; Guo et al., 2001). Troncoso-Rojas et al. (2013) use ISSR and RAPD makers to evaluate the genetic diversity and genotyping of Alternaria solani isolates. DNA markers such as RFLP, AFLP, RAPD, ISSRs, simple sequence repeats (SSR), and expressed sequence tags (EST) were used in the analysis of the genetic variation in various F. oxysporum formae speciales (Bogale et...
al., 2005; Dubey and Singh 2008; Dubey et al., 2010; Sharma et al., 2014; Yuan et al., 2013). Singh and Kapoor (2018) used ISSR markers to develop sequence-characterized amplified region (SCAR) markers to identify and quantify *Fusarium oxysporum* f. sp. *carthami*. Molecular tools are the most accurate for identifying plant pathogens. Their use has increased in recent years due to the availability of specific equipment and chemicals and their ability to identify the types and aggressiveness of isolates (Adss et al., 2017). This study aimed to isolate, purify and identify *F. oxysporum*. f. sp. *lycopersici* isolates from six governorates of Egypt and differentiate these isolated using ISSR–PCR and virulence test.

### MATERIALS AND METHODS

1. **The causal fungal sample collections**

Eight fungal isolates were isolated from infected tomato plants with typical wilt symptoms collected from six governorates in Egypt; El-Menofia (Talla and Shebeen ElKom), El-Menia, Assiut, Kafr El-Sheikh, Alexandria, El-Beheira (Abou-Homus and Kom Hamada).

2. **Isolation and identification of the causal fungi**

The collected tomato root rot samples were thoroughly washed in tap water and air-dried. Small pieces were taken from the internal tissues adjacent to the affected root parts, surface sterilized in 3% sodium hypochlorite solution for 3 mins., rinsed in sterile distilled water, and dried between sterilized filter papers. After that, samples were transferred to PDA plates containing streptomycin sulphate (0.2g/L) and incubated at 28°C for five days (Shaukat et al., 2005). The hyphal tip technique was used to purify the isolated fungi, as described by Brown (1924). Identifications of the causal fungi were made at the Department of Plant Pathology, Faculty of Agriculture, Damanhour university, according to Gilman (1957), Singh (1982), and Summerrell et al. (2003). The frequency of the fungal isolates recovered from the surveyed governorates was calculated.

3. **Pathogenicity test**

The eight *F. oxysporum* f. sp. *lycopersici* isolates were tested for pathogenicity on the Carmen tomato cultivar. Tomato seeds were sterilized for five min. in 1% sodium hypochlorite, rinsed with sterile water, and sown in 20cm diameter plastic pots filled with 3Kg sterilized peat moss, clay, and sand (1:1:1). For Fungi inoculum preparations, pathogens were cultured on Potato Dextrose Broth (PDB) medium in 250ml conical flasks and incubated at 25 ± 2°C for 10 days. The mycelial cultures were then filtered, and the spore suspensions were adjusted to 10^6 conidia/ml using a haemacytometer slide. Plants were inoculated when they reached 15-20cm tall by adding 50 ml of the prepared conidial suspension to 1kg of soil (Elad and Baker, 1985).

4. **Disease assessments**

The severity of wilting was rated 15-day post-inoculation (dpi) using a pathogenicity scale of 6 grades, according to Liu et al. (1995) (Table 1).

<table>
<thead>
<tr>
<th>Category</th>
<th>Grade</th>
<th>% of Infected Leaf Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>no symptoms</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>Plants with &lt; 25% of diseased leaves</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>Plants with 25-50% of diseased leaves</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>Plants with 50-75% of diseased leaves</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>Plants with 76-100% of diseased leaves</td>
</tr>
<tr>
<td>VI</td>
<td>5</td>
<td>Plants completely died.</td>
</tr>
</tbody>
</table>

5. **Differentiation between *F. oxysporum* isolates using ISSR analysis**

5.1. **Fungal DNA extraction**

Fungal genomic DNA was isolated by Wizard Genomic DNA purification kit (Promega, Germany) according to the manufacturer’s procedures.

5.2. **ISSR reactions**

PCR amplification was performed using nine ISSR primers (Table 2) to differentiate and fingerprint the *F. oxysporum* isolates. PCR reaction (25µl) contained 10x PCR buffer (2.5µl), 4mM dNTPs (2.5µl), 50mM MgCl2 (2.5µl), 50pmol primer (7µl), 50ng of fungal genomic DNA (1µl), and 5 units/µl Taq DNA polymerase (0.2µl) (Promega, Germany). The PCR program was: initial denaturation at 95°C for 5 min, then 40 cycles of 95°C for 1 min, annealing at a temperature depending on primer for 1 min (Table 2), extension at 72°C for 1 min, and a final extension at 72°C for 10 min (Istock et al., 2001). The amplified DNA products were electrophoresed on 1.5% (w/v) agarose gel in 0.5 x TBE buffer, stained with ethidium bromide (0.5 µg/cm3, w/v), and photographed using a gel documentation system.
5.3. Construction of dendrogram based on ISSR-PCR DNA banding patterns.
ISSR-PCR banding patterns for each primer were recorded depending on the presence and absence of the amplified bands. A band is scored as (1) or (0), depending on whether it is present or absent. Multi-Variate Statistical Package (MVSP) Version 3.1 was used to compare the *F. oxysporum* isolates and the unweighted pair group method with arithmetic averages (UPGMA) used to compute the similarity coefficients and to conduct cluster analysis.

6. Statistical analysis
Disease severity data were analyzed with SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA), and the least significant difference (LSD) test was used to differentiate means at a p ≤ 0.05.

RESULTS AND DISCUSSIONS
*F. oxysporum*, f. sp. *lycopersici* is the disease agent of tomato fusarium wilt and one of the most critical tomato pathogens, and it causes significant damage and economically severe crop losses, particularly at high temperatures and humidity in the indoor environment (Jones et al., 1982; Smith et al., 1988; Agrios, 2005).

1. Isolation and Identification of *F. oxysporum* isolates

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence (5′ to 3′)</th>
<th>Annealing Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB-09</td>
<td>GTGTGTGTGTGTGG</td>
<td>55</td>
</tr>
<tr>
<td>HB-11</td>
<td>GTGTGTGTGTGTGCC</td>
<td>52</td>
</tr>
<tr>
<td>HB-12</td>
<td>CACCAACACACGC</td>
<td>51</td>
</tr>
<tr>
<td>HB-13</td>
<td>GAGAGAGAGAGC</td>
<td>50</td>
</tr>
<tr>
<td>HB-14</td>
<td>GTGTGTGTGTGTGCC</td>
<td>55</td>
</tr>
<tr>
<td>UBC814</td>
<td>ACACACACACACACACC</td>
<td>55</td>
</tr>
<tr>
<td>UBC835</td>
<td>AGAGAGAGAGAGAGAGAGAGAGAGAC</td>
<td>60</td>
</tr>
<tr>
<td>UBC840</td>
<td>GAGAGAGAGAGAGAGAGATT</td>
<td>55</td>
</tr>
<tr>
<td>UBC868</td>
<td>GAAGAAGAAGAAGAAGAAGA</td>
<td>51</td>
</tr>
</tbody>
</table>

Eight isolates of *F. oxysporum* (FOT) were obtained and identified from tomato-infected plants (Table 3) collected from El-Menofia (Talla (FOT1) and Shebeen Elkom (FOT2)), El-Menia (FOT3), Assiut (FOT4), Kafr El-Sheikh (FOT5), Alexandria (FOT6), El-Beheira governorate (Abou-Homus (FOT7) and Kom Hamada (FOT8)).

2. Pathogenicity test
The eight *F. oxysporum* isolates were tested for pathogenicity on the highly susceptible tomato cultivar Carmen. Data presented in Table (3) and Fig (1) showed that all eight isolates were virulent. Isolates FOT4 from Assiut, FOT2 from El-Menofia (Shebeen Elkom), and FOT5 from Kafr El-Sheikh were highly virulent. In comparison, isolates FOT6 from Alexandria and FOT8 from El-Beheira (Kom Hamada) were moderately virulent. While isolates FOT7 from Abou Homus, FOT3 from El-Menia and FOT1 from El-Menofia (Talla) were weakly virulent. These results agreed with those of Abou-Zeid *et al.* (2016). They obtained six *F. oxysporum* isolates from diseased pepper collected from Gharbeya and Dakahlia governorates in Egypt. The F3 isolate from Dakahlia was more aggressive (96.67% wilt severity) than Tanta F4 isolate (71.67% severity).

Table 3. The pathogenicity test of eight *F. oxysporum*, f. sp. *lycopersici* isolates on Carmen tomato cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease severity* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOT4</td>
<td>75e</td>
</tr>
<tr>
<td>FOT2</td>
<td>70e</td>
</tr>
<tr>
<td>FOT5</td>
<td>68a</td>
</tr>
<tr>
<td>FOT6</td>
<td>50b</td>
</tr>
<tr>
<td>FOT8</td>
<td>46b</td>
</tr>
<tr>
<td>FOT7</td>
<td>30c</td>
</tr>
<tr>
<td>FOT3</td>
<td>25c</td>
</tr>
<tr>
<td>FOT1</td>
<td>13d</td>
</tr>
</tbody>
</table>

*Data are an average of three replicates. LSD_{0.05} for fungal isolates = 8.24
3. Fingerprinting of *F. oxysporum* isolates using ISSR markers

3.1. ISSR banding pattern analysis

Genetic diversity analysis of the eight *F. oxysporum* isolates using ISSR markers revealed a high variability level among these isolates. The nine tested ISSRs primers (UBC814, UBC840, UBC808, UBC811, UBC868, HB-11, HB-12, and HB-13) were polymorphic, and successfully generated 81 DNA loci with a molecular size ranging between 130-1500bp (Fig. 2). DNA banding pattern analysis showed 37 monomorphic and 44 polymorphic bands, giving 54.32% polymorphism among the tested isolates. Primer UMC-840 had the highest level of polymorphism (85.71%) among the tested isolates, while primer HB-13 had the lowest level (33.33%).

Primer UBC814 yielded 57 bands found in nine loci with molecular sizes ranging between 150-1400bp. Five of the nine observed loci were polymorphic, with 55.55% Polymorphism between isolates. This primer provided the lowest numbers of bands (6 bands) with isolates FOT3, FOT5, and FOT8, while the highest numbers (9 bands) were observed with FOT4 isolates. Primer UBC840 generated 37 fragments expressed in seven loci ranging from 160 to 1400bp. Six of these loci were polymorphic, and the highest level of polymorphism among the isolates (85.71%) was observed with this primer.

Primer UBC868 produced 54 fragments presented in nine loci with molecular weights ranging between 150-1000bp. Five of the nine detected loci were polymorphic, with 55.55% polymorphism. This primer yielded the lowest numbers of bands (6 bands) with FOT7, FOT5, FOT6, and FOT4 isolates, while it produced the highest (8 bands) with FOT1 and FOT2 isolates. Primer UBC835 generated 48 fragments phrased in 9 loci with different molecular sizes (130-1500bp). The polymorphism level of this primer was 77.77%, with seven of the nine observed loci being polymorphic. The lowest numbers of derived bands (5 bands) were noted with isolates FOT6, while the highest numbers (9 bands) were noted with the FOT7 isolate.

Primer UB-11 produced 47 bands expressed in nine loci with molecular weights ranging from 200 to 1200bp. This primer had a polymorphism level of 55.55% with five polymorphic loci. The highest number of obtained bands (8 bands) were detected with the FOT7 isolate, while the lowest number (5 bands) were detected with FOT1 and FOT6 isolates. Primer UB-13 generated 65 fragments in nine loci ranging from 130 to 970bp. Three of the nine observed loci were considered polymorphic, and the lowest level of polymorphism among the tested isolates (33.33%) was observed with this primer. Isolates FOT1 and FOT4 had the lowest numbers of generated bands (7 bands), while isolates FOT2, FOT3, and FOT7 had the highest (8 bands).

Primer UB-09 amplified 48 fragments in nine loci with molecular weights ranging between 150 and 900bp. Five of the nine detected loci were polymorphic, with 55.55% polymorphism. This primer generated five bands with FOT6 isolate and eight bands with FOT7 isolate. Primer UB-12 yielded 52 bands in eight loci with molecular sizes ranging from 180 to 970bp. Four of these observed loci were considered polymorphic. The level of polymorphism among the tested isolates was 50%. This primer produced five bands with isolates FOT7, while it produced eight bands with FOT4 isolate. Primer UB-14 generated 35 fragments in six loci with molecular sizes ranging between 190 and 700bp. Three of the six detected loci were polymorphic, with a polymorphism level of 50%. Three bands were recorded with the FOT1 isolate, while six bands were recorded with the FOT4 isolate.

These findings are consistent with previous research that found ISSR markers to be helpful in assessing the genetic variability of *F. oxysporum* (Bayraktar et al., 2008; Dubey and Singh, 2008; Baysal et al., 2010; Thangavelu et al.,
ISSR markers produce higher polymorphism because they amplify the conserved regions between the microsatellite repeat sequences, which are widely distributed in the genome (Zietkiewicz et al., 1994). Differences in ISSR polymorphism were also found in Fusarium strains isolated from either cabbage or tomato (Troncoso-Rojas et al., 2013). Singh et al. (2019) reported high genetic diversity in 90 isolates of *F. oxysporum f. sp. carthami* isolated from Safflower using 17 polymorphic RAPD and ISSR primers.


The dendrogram presented in Fig. (3) revealed that the eight tested isolates were divided into Three main clusters (A, B, and C). Cluster A is divided into two sub-clusters; sub-cluster A1 is split into two groups, group1 contains two subgroups, subgroup1 includes FOT6 isolate, and subgroup 2 includes FOT4, FOT3, and FOT5 isolates (FOT4 and FOT5 are highly virulent isolates), group2 contains FOT8 isolate. Subcluster A2 includes FOT6 isolate. However, cluster B includes the FOT1 isolate, while cluster C contains the FOT2 isolate.

Cluster analysis using ISSR markers showed that the isolates were distributed in
different groups, indicating that *F. oxysporum* has high genetic variability. These findings are in harmony with that detected by Bayraktar et al. (2008). They found that 74 isolates of *F. oxysporum* obtained from 13 regions in Turkey were divided into three major groups using ISSR, RAPD, and virulence analysis. ISSR and RAPD were used to classify *F. oxysporum* *f. melongenae* isolates into several groups, revealing high levels of genetic specificity and variability, implying that genetic variations are associated with the fungus in the Mediterranean region (Baysal et al., 2010). Cluster analyses assigned isolates of *F. oxysporum* *f. sp. carthami* from various Indian states to different clusters, reflecting a high level of genetic variations (Singh et al., 2019). Because *F. oxysporum* has no known sexual stage, the generation of new genetic recombinations does not result from meiotic crossing over (Booth, 1971; Gordon and Martyn, 1997). Instead, other mechanisms, including mutations, genetic drift, transposable elements, selection, gene flow, and hyphal anastomosis, are thought to drive species evolution (McDonald and Linde, 2002). Bayraktar et al. (2008) cleared that the low-genetic variation and high gene flow between populations influenced the *F. oxysporum* *f. sp. Ciceris* formation and evolution.

**Fig. 3.** The dendrogram shows the phylogenetic relationship among the eight *F. oxysporum* based on ISSRs analysis.

**Table 4.** A similarity matrix between the tested *F. oxysporum* isolates based on ISSR band pattern analysis and Jaccard index.

<table>
<thead>
<tr>
<th></th>
<th>FOT7</th>
<th>FOT1</th>
<th>FOT3</th>
<th>FOT5</th>
<th>FOT6</th>
<th>FOT2</th>
<th>FOT8</th>
<th>FOT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOT7</td>
<td>100</td>
<td>64</td>
<td>68</td>
<td>64.8</td>
<td>68</td>
<td>70</td>
<td>67.4</td>
<td>64</td>
</tr>
<tr>
<td>FOT1</td>
<td>100</td>
<td>63.6</td>
<td>65</td>
<td>67</td>
<td>63.5</td>
<td>67.6</td>
<td>66</td>
<td>63.5</td>
</tr>
<tr>
<td>FOT3</td>
<td>100</td>
<td>79</td>
<td>79</td>
<td>64</td>
<td>67.6</td>
<td>75</td>
<td>82.6</td>
<td>75</td>
</tr>
<tr>
<td>FOT5</td>
<td>100</td>
<td>81</td>
<td>67.3</td>
<td>67.6</td>
<td>75</td>
<td>81.6</td>
<td>82.6</td>
<td>78.5</td>
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<tr>
<td>FOT6</td>
<td>100</td>
<td>70.8</td>
<td>72.3</td>
<td>78.5</td>
<td>72.3</td>
<td>78.5</td>
<td>78.5</td>
<td>78.5</td>
</tr>
<tr>
<td>FOT2</td>
<td>100</td>
<td>70.6</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>FOT8</td>
<td>100</td>
<td>76.6</td>
<td>76.6</td>
<td>76.6</td>
<td>76.6</td>
<td>76.6</td>
<td>76.6</td>
<td>76.6</td>
</tr>
<tr>
<td>FOT4</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The findings of this study revealed a high level of variation between *F. oxysporum*, the causative agent of the tomato Fusarium wilt, using ISSR and virulence analysis. Both approaches were effective in analyzing the populations of *F. oxysporum* isolates. ISSR markers proved to be remarkably informative for studying the polymorphisms in *F. oxysporum* populations. One of the priority goals should be to use molecular markers to learn more about the molecular evolution of *F. oxysporum* that infects tomato plants in several Egyptian governorates, which is critical for breeding tomato against Fusarium wilt.

**REFERENCES**


Thangavelu, R., K. M. Kumar, P. G. Devi and M. M. Mustaffa. 2012. Genetic diversity of Fusarium oxysporum f. sp. cubense isolates (Foc) of India by inter simple sequence repeats (ISSR) analysis. Molecular Biotechnology, 51:203-211.


الملخص العربي

التفريق بين عزلات الذبول الفيوزرايوم فى الطماطم باستخدام معلمات ISSR markers

وختبارات المرضية

سوسن صلاح الدين الشامى 1 وأبراهيم أحمد عدس 2 و غنيم محمد عامر 1


يعتبر فطر فيوزرايوم أوكسيسبوروم يسبب الذبول الفيوزرايوم في الطماطم، وهو أحد الأمراض الفطرية الخطيرة التي تسبب خسائر كبيرة في إنتاج الطماطم في مصر. تم عزل ثماني عزلات من F. oxysporum من نباتات طماطم مصابة شديدة المرضية، بينما كانت العزلات FOT4 و FOT5 و FOT2 و FOT3 من مست محافظات مصرية. كنتم العزلات FOT6 و FOT8 ضعيفة المرضية، وكانت FOT1 و FOT7 Mتوسطة المرضية. كانت جميع بادئات ISSRs المختبرة متعددة الأشكال وأنتجت 81 موقع DNA بأحجام جزيئية مختلفة. أظهر تحليل أنماط شظايا الحمض النووي 37 نطاقًا أحادي polymorphism و 44 حزمة متعدد الأشكال monomorphic من بين العزلات المختبرة كان لبادئ UMCI-840 (أعلى مستوى من تعدد الأشكال UMC-85.71%) ، بينما كان لبادئ HB-13 (أقل مستوى 33.33%). تم تحليل أنماط UPGMA و Dendrogram باستخدام طريقة UPGMA و Dendrogram لاكتشاف العلاقة بين العزلات الثمانية. ونجد أعلى تكاثر وراثي (98.2%) بين عزلات FOT4 و FOT3 و FOT1 و FOT2. بينما لم نلاحظ أقل تكاثر وراثي (63.5%) بين عزلات FOT6 و FOT8. تم تصنيف العزلات الثمانية إلى ثلاث عناقيد رئيسية A و B و C. تحت عناقيد A المجموعتين تحت FOT1 و FOT2. تحت عناقيد B المجموعتين تحت FOT4 و FOT5 و FOT6. تحت عناقيد C المجموعتين تحت FOT8. في عناقيد A: العزلة FOT6 تحت العنقود A1 و FOT1 تحت العنقود B و FOT2 تحت العنقود C. في عناقيد B: العزلة FOT3 تحت العنقود A2 و العزلة FOT5 تحت العنقود A3 و العزلة FOT4 تحت العنقود A4 و العزلة FOT2 تحت العنقود A5 و العزلة FOT3 تحت العنقود A6 و العزلة FOT5 تحت العنقود A7 و العزلة FOT6 تحت العنقود A8 و العزلة FOT8 تحت العنقود A9 و العزلة FOT4 تحت العنقود A10.

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