Geographical Distribution, Identification and Control Methods of Citrus Tristeza Virus and Its Effects on Citrus Production

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Abstract: Citrus is one of the most economically important fruit crops grown by smallholders and commercial farmers in Ethiopia. Citrus production and productivity in Ethiopia is seriously threatened by various diseases including citrus Tristeza Virus. Therefore, the aim of this review is to access the production status and possible control measures of Citrus Tristeza Virus under Ethiopian condition. Citrus is among the most important fruit crops in Ethiopia. Its cultivation started in Upper Awash valley and Melkassa areas in central Ethiopia. Citrus Tristeza Virus (CTV) epidemiology is significantly affected by the citrus cultivar and horticultural practices the most important factors are the Citrus Tristeza Virus isolate and the aphid vector. Morphologically the virus is small sized with rod. Since the virus is phloem-limited, most Citrus Tristeza Virus symptoms are associated with viral disruption of phloem and its function. Natural spread of the virus is primarily through propagation of infected bud wood and by aphids but is not seed-borne. Control strategies for virus differ according to the incidence and severity of the Citrus tristeza virus isolates in an area and with the cultivars and root stocks used. No single control strategy is applicable in all situations. Preventive efforts should be made to avoid introduction of Citrus Tristeza Virus into the growing area by having quarantines on importation of live citrus tissue. The virus control strategies should include exclusion of the pathogen, application of planting material certification, total eradication and suppression of diseased plant, using resistant/tolerant root stock, tolerant scions, crossprotection, vector control and integrated disease management are possible control methods. Therefore, the development of integrated disease management is best and environmentally friendly alternative for practices for the management of virus and can go a long way in maintaining a sustainable environment.

Keywords: Citrus, control, disease, fruit, virus

1. INTRODUCTION

Citrus (Citrus spp.) is one of the most economically important fruit crops grown by smallholders and commercial farmers in Ethiopia (Kassahun et al., 2006; Yesuf, 2007). The total area coverage and the annual production of citrus were estimated 5165.28 ha and 38486.5 million tons respectively (CSA, 2018). Ethiopia is a relative small newcomer in citrus trade (Seifu, 2003) and Ethiopia had been known to export citrus from the 1960’s to the Middle East and Western Europe. However, over the past 30 years export volumes have dropped due to poor quality delivered onto the market, which is mainly due to lack of improved production practices and technology transfer has hampered industry growth (Harris, 1985). In addition, citrus production and productivity in Ethiopia is seriously threatened by various diseases including citrus Tristeza (Derso, 1999; Yesuf, 2007; Mekbib, 2007) which is caused by from one generation to the next and also from one country to another in the absence of strict and citrus Tristeza virus. Citrus is mainly propagated by grafting. Therefore, Tristeza virus is passed on enforced phytosanitary and quarantine regulations. A variety of graft-inoculation methods are used to experimentally transmit the virus (Abdelselam, 2002). Tristeza apparently originated in Asia and existed there for many years in tolerant cultivars which were either propagated vegetatively as cuttings or by seed. Farmers in Brazil and other South American countries gave it the name "tristeza", meaning sadness in Portuguese and Spanish, referring to the devastation produced by the disease in the 1930s. The virus is transmitted most efficiently by the brown citrus aphid. Tristeza citricida is the most efficient vector and where it exists, is often the most abundant aphid on citrus (Yokomi et al., 1994). Citrus Tristeza Virus (CTV) epidemiology is significantly affected by the citrus
cultur and horticultural practices the most important factors are the CTV isolate and the aphid vector.

Major symptoms are wilting, chlorosis and an abnormal crop forming small fruit which may persist after tree death. Clinical symptoms can often be seen by removing a patch of bark across the bud union. Trees which decline slowly will have thicker bark immediately below the union and the face of the bark surface below the union will have many small conical pits (honey combing) corresponding to bristle-like protuberances from the wood (Schneider,1954). The aim of this review is to access the production status and possible control measures of Citrus Tristeza Virus under Ethiopian condition.

2. PRODUCTION STATUS AND IMPORTANCE OF CITRUS TRISTEZA VIRUS

2.1. Importance Citrus Tristeza Virus on Citrus Fruit

Citrus is among the most important fruit crops in Ethiopia. Its cultivation started in Upper Awash valley and Melkassa areas in central Ethiopia (Kassahun et al.,2006). Virus problems (13.6%), prevalence of nematodes (12.1%) and mole rats (1.5%) were also reported to cause dieback at Error Gota, Toni, Hursso and Tisablalima farms (Mekbib,2007). Citrus tristeza virus is the most economically important virus pathogen of citrus worldwide. The disease destroys millions of trees throughout the world. It occurs in most citrus-producing areas and represents a very serious threat to the citrus industry of the Mediterranean Basin (Djelouah and D’Onghia,2001). Millions of citrus trees on sour orange have been killed by citrus tristeza decline epidemics in Argentina, Brazil, Venezuela, Peru, Florida, California, Israel, Spain, and other locations including Ethiopia. In addition citrus production and productivity in Ethiopia is seriously threatened by various diseases including citrus Tristeza (Derso,1999;Yesuf,2007;Mekbib,2007) which is caused by citrus Tristeza virus. In addition to decline, many severe CTV isolates cause stem pitting diseases of susceptible scions cultivars and these occur even when tolerant root stocks are used. Stem pitting weakens trees and eventually reduces fruit size, quality, and quantity (Marais et al., 1996). Grapefruit and lime are very sensitive to stem pitting. Sweet orange is more tolerant but can be severely affected by some virus isolates. Some isolates of CTV cause a decline when field trees of sweet orange, mandarins, or grapefruit grafted on sour orange root stocks become infected. This decline is associated with a virus induced phloem necrosis at the bud union which blocks normal translocation of carbohydrates to the root system. As the root system deteriorates, trees begin to decline.

2.2. Morphology of Virus and its Characteristics

Citrus tristeza virus particles are flexuous rods 10-11 nm in diameter and 2,000 nm long. The particles are easily sheared and extracts from partially purified preparations typically contain many broken particles of various lengths. Plant viruses come in different shapes and sizes. Nearly half of them are elongate rigid rods or flexuous threads and almost as many are spherical (isometric or polyhedral) with the remaining being cylindrical bacillus-like rods.

The capsid protein sub units are helically arranged along the particle with a basic pitch of 3.7 nm and ten sub units in each turn of the helix (Bar-Joseph et al.,1972). The nucleic acid is single-stranded positive sense RNA with a molecular weight of about 6.5 x 10^6 which is composed of 19,296 nucleotides in the isolate T36 from Florida or 19226 nucleotide in the isolate VT from Israel (Mawassi et al.,1996). These encode 12 open reading frames (ORF) potentially coding for at least 17 protein products. Nine 3’ co-terminal subgenomic RNAs have been found in infected citrus tissue (Hilf et al.,1995).

Sequence comparisons between strains are in progress and indicate a relatively high level of conservation toward the 3’ end and divergence up to 40 % toward the 5i terminal (Mawassi et al.,1996). Many CTV- infected plants contain defective RNA's which are composed of portions of the 5’ and 3’ terminal sequences of the CTV genome (Mawassi et al.,1996). Some of the defective RNAs have been found to contain small portions of non-virus encoded RNA linking the 5’ and 3’ terminal portions. Defective RNAs are often detected as prominent bands in purified extracts of double stranded RNA from infected tissues. The significance of defective RNAs on symptom expression has not been determined (Mawassi et al.,1996).
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2.3. Symptoms

Being phloem-limited, most citrus tristeza virus symptoms are associated with viral disruption of phloem and its function. Some isolates cause few symptoms even in plants that are normally reactive such as Mexican lime (Bové et al., 1988). Most CTV strains cause vein licking, leaf cupping, a transient leaf epinasty in young leaves and some stem pitting on CT V-sensitive plants such as Mexican lime, Citrus macrophylla, or Citrus hystrix. These vary from mild to severe and impact fruit quality when severe.

Symptoms appear a year or more after infection and may occur gradually over several years or very suddenly. Canopy symptoms are wilting, chlorosis and an abnormal crop of small fruit which may persist after tree death in trees affected by CTV. Clinical symptoms can often be seen by removing a patch of bark across the bud union.
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Figure 3. Xylem blockage that is associated with blight.

Trees which decline slowly usually will have thicker bark immediately below the union and the face of the bark surface below the union will have many small conical pits (honey combing) corresponding to bristle-like protuberances from the wood (Schneider, 1954). If bud woods from scions infected with decline strains of CTV are propagated on sour orange seedlings, the budtings may be stunted and chlorotic but rarely collapse and die (Bransky et al., 1986). Stem pitting does not kill trees but affected trees may have thin canopies and produce fewer fruit of reduced size and quality (Marais et al., 1996). Die back occurs in severely affected limes. Some strains of CTV also induce a seedling yellows (SY) reaction (adwaring and general chlorosis) in inoculated grapefruit, lemon, and sour orange seedlings. The SY reaction usually but not always accompanies the presence of decline on sour orange and/or stem pitting strains of CTV.

2.4. Hosts Range
Citrus tristeza virus infects all species, cultivars and hybrids of citrus. Natural hosts for citrus tristeza virus include nearly all citrus species inter specific hybrids, some citrus relatives and some inter generic hybrids. The only natural non citrus host that has been reported is Passilora (Kitajima et al., 1974). Some of the more important economic hosts are: sweet orange, grape fruit, mandarins, limes and lemons.

2.5 Taxonomic Classification
CTV is a member of the closterovirus group which have thread-like, lexuousvirions, insect vectors and cause characteristic cytopathological structures (inclusion bodies) in infected phloem tissues and have a positive sense single-stranded RNA genome of up to 20 kb. Based on molecular characterization of CTV and other members of the closterovirus group enough genetic diversity occurs among members to propose that the closteroviruses belong to the family Closteroviridae which contains homologues of cellular heat-shock proteins (Dolja et al., 1994). The Closteroviridae are composed of three genera: Citrivirus (CTV = type member) which has one 19.3 kb genome component and 12 open reading frames (ORFs); Closterovirus (beet yellows virus = type member) which has one 15.4 kb genome and 9 ORFs; and Biclovirus (lettuce infectious yellows = type member) which has two genome components of 8.1 and 7.1 kb and 9 ORFs.

2.6. Geographical Distribution
Tristeza apparently originated in Asia and existed there for many years in tolerant cultivars which were either propagated vegetatively as cuttings or by seed. New areas of citriculture in other continents were first established from seed and were free of CTV infection. Subsequently, CTV has been introduced into nearly all citrus-growing areas via virus-infected bud wood or plants. In many areas infections have become wide spread due to propagation and secondary spread by aphids. In some areas little or no secondary spread has occurred from the few existing infected trees.

CTV is very common in commercial citrus in southeast Asia, Australia, southern Africa, India, Japan, South America, and most Pacific Islands and where the brown citrus aphid is present nearly all field-
grown trees are infected. CTV is widespread in parts of Spain, Florida, parts of California, portions of Central America and most of the Caribbean islands. CTV is present but not widespread in parts of the Mediterranean region and Mexico.

2.7. Biology and ecology of Citrus Tristeza Virus

2.7.1. Transmission Mechanism

Natural spread of CTV is primarily through propagation of infected bud wood and by aphids. CTV is not seed-borne. Propagation of new plants from buds from infected plants is responsible for long-distance spread of CTV and extensive bud propagation from a single plant can rapidly increase foci of inoculum. Top working old citrus plantings to new scion varieties using infected buds has been a common way of CTV spread in some countries. Tree to tree spread is by aphids. CTV is semi persistently transmitted by several aphid species (Bar-Joseph et al., 1989). Aphid vectors acquire the virus from an infected tree with feeding times ranging from 5 minutes to hours. However, it is not by brief probes. The transmission efficiency of the vector increases as the acquisition and feeding times are increased up to 24 hours. There is no latent period and the virus does not multiply or circulate in the aphid. The time required to inoculate a plant is the same as for acquisition. Aphids remain viruliferous for 24-48 hours after feeding on infected plants.

Many aphids species that feed on an infected citrus tree can acquire CTV as detected by ELISA (Cambra et al., 1982) but only a few species can transmit it to new plants. T. citricida is the most efficient vector and where it exists, is often the most abundant aphid on citrus (Yokomi and Tang, 1994). Most isolates of CTV including severe stem pitting isolates are effectively vectored by T. citricida but a few isolates are vectored less efficiently. Aphis gossypii Glover (melon or cotton aphid) can transmit some isolates efficiently and is the most important CTV vector in regions where T. citricidas not present (Mendoza et al., 1984; Yokomi and Tang, 1996; Ballester et al., 1993). In contrast to T. citricida, it has a wide host range and only occasionally colonizes citrus. Toxoptera aurantii can transmit some CTV isolates but is less efficient than brown citrus aphid or melon aphid (Mendoza et al., 1984). It also has a wide host range and occasionally colonizes citrus. Aspiraecola Patch (spirea aphid) is an inefficient vector of CTV under experimental conditions but is very common on citrus worldwide and also is extremely polyphagous (Mendoza et al. 1984; Yokomi and Tang, 1996). These aphids except T. citricida are distributed worldwide. Several other aphids have been shown to transmit CTV experimentally but are not likely to be significant.

CTV is readily graft transmissible if a union is formed between the phloem of the donor and receptor host. A variety of graft-inoculation methods are used to experimentally transmit the virus (Abdeselam, 2002). Buds sections of leaves that include veins and stem pieces can all be used as inoculum. Mechanical transmission of CTV is difficult and has only been done experimentally by slash-inoculation of the stems of receptor plants with concentrated extracts from CTV-infected plants. CTV can also be transmitted experimentally by dodder.

2.8. Epidemiology

Primary infections of CTV are usually established via propagation of infected plants. Epidemics of CTV decline observed in many countries began with importation and propagation of infected plants in areas heavily planted with CTV-free trees on sour orange. When efficient vectors were present epidemics of decline often followed. Although CTV epidemiology is significantly affected by the citrus cultivar and horticultural practices, the most important factors are the CTV isolate and the aphid vector. When T. Citricidas present, temporal and spatial spread of CTV spread is increased (Garnsey et al., 1996a). This aphid has a narrow host range and migrants move from citrus to citrus to start new aphid colonies and in this process, can transmit CTV if they are viruliferous. High aphid populations also coincide with new lush which is favorable for virus acquisition and inoculation. The other vectors are much less efficient than T. citricida and also have a wider host range. Migrants may originate in other crops prior to feeding on citrus and may feed on a different plant species after leaving citrus. Therefore, aphid host range and feeding behavior likely affect pattern and rate of spread (Gottwald et al., 2002). It is assumed that aphid population levels may be correlated with rates of spread but threshold levels for minimum and maximum levels of transmission have not been established. Natural spread is generally slow in desert regions where natural thermotherapy may keep inoculum at lower
levels in plants and may vary seasonally in temperate areas as well. CTV spread rate in sweet oranges is generally higher than that observed in grapefruit (Menzola et al., 1984; Gottwald et al., 2002). CTV isolates in Meyer lemon and some mandarins have not spread appreciably from these hosts unless T. citricidas present. The latent periods between inoculation and systemic infection between infection and symptom expression also affect evaluation of disease development. Presence of other strains may also influence rate of virus movement (Mendoza et al., 1984).

2.9. Control Measures

Control strategies for CTV differ according to the incidence and severity of the CTV isolates in an area and with the cultivars and root stocks used. No single control strategy is applicable in all situations (Garnsey et al., 1996a).

2.9.1. Exclusion and Quarantine

When CTV is absent or rare, preventive efforts should be made to avoid introduction of CTV into the growing area by having quarantines on importation of live citrus tissue. A practical and safe method to legally introduce cultivars from other regions and to free these of infection is necessary and reduces industry pressure to illegally introduce new cultivars or germplasm resources. Procedures for safe international movement of citrus germplasm have been devised (Frison and Taher, 1991).

2.9.2. Certification Programs

Careful control of propagating material remains the single most effective means to avoid rapid And extensive CTV epidemics. Most commercial citrus are clonally propagated by using buds from a selected scion cultivar to a nucellar seedling as a root stock. Bud wood is usually taken from mature, vigorous tree and used directly or increased in a nursery block to produce thousands of buds from a single source. Thus, propagation of CTV infected trees can be prevented by using virus-free scion trees protected from natural infection by isolation or use of insect free screen houses or by shoot tip grafting (Navarro, 1993).

2.9.3. Eradication and Suppression

If a few trees become infected in a CTV-free area and indigenous aphids are poor vectors, natural spread can be slowed appreciably by a vigilant eradication and suppression program. However, an effective survey program is essential and when CTV is detected, infected trees must be removed immediately and surveillance maintained (Garnsey et al., 1996). Eradication is rarely effective once infections are well established especially in the presence of favorable vector conditions.

2.9.4. Resistant/Tolerant Root Stocks

Numerous root stocks are tolerant or resistant to CTV decline and use of these is essential for economic production of citrus in many areas. Some examples are Cleopatra and Sunki mandarins, rough lemon, Rangpur lime, trifoliate orange, and trifoliate orange hybrids such as Troyer and Carrizo citranges and Swingle citrumelo. CTV resistant/tolerant root stocks are often susceptible to other problems such as citrus blight, viroids, nematodes, or poor soil conditions.

2.9.5. Using Tolerant Scions

Most mandarins are generally tolerant to CTV, although some hybrids, such as some tangelos, are seriously affected by stem pitting. In most areas in Asia where CTV isolates are severe, mandarins are the principal varieties produced due in part to their tolerance to stem pitting. There are no CTV-tolerant limes although Persian limes are more tolerant than small acid limes. All grapefruits are susceptible to grapefruit stem pitting isolates of CTV. Sweet oranges vary in susceptibility to sweet orange stem pitting but none are truly tolerant. Pera orange, a major variety in Brazil, is very susceptible while Valencia is one of the more tolerant cultivars.

2.9.6. Cross Protection

Infection with a mild isolate of CTV may protect a tree from becoming infected with or showing Symptoms of a more virulent strain CTV (Gottwald et al., 1989). This is a strategy for control of stem pitting in areas where severe isolates of CTV and the brown citrus aphid are endemic. Cross
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Protection is for production of grapefruit in South Africa (Navarro et al., 1993) and Pera sweet orange and Galego lime in Brazil. In these countries, protective isolates have been selected from vigorous trees that remained in areas destroyed by the disease and their protective capacity confirmed in controlled experiments. Protection often is effective only between certain isolates and many mild isolates show little protective effect (Navarro et al., 1993). Furthermore, mild protective isolates are often effective only in the specific cultivar in which they were selected. Effective long-term cross protection against decline of trees grafted on sour orange root stock has not been demonstrated, though significant delay of symptom onset has been observed with some mild isolates (Yokomi and Tang, 1999). Cross protection is an empiric practice and the basis for the strain interaction involved is not understood. One of the difficulties to implement an effective cross protection is that many CTV isolates contain a mixture of strains that differ at molecular level and in symptom expression (Moreno et al., 1993). The balance of strains in a CTV isolate may change depending on the host and other factors and, hence, its specific interactions with other isolates. Therefore, until additional understanding on the molecular mechanism involved in this interaction will be available, cross protection has to be considered as a practical procedure to delay or reduce damage caused by severe isolates in some citrus cultivars grown in specific areas.

2.9.7. Vector Control

Vector suppression is an unproven strategy for CTV control. In the case of semi persistently transmitted viruses, viruliferous winged aphids may inoculate citrus trees several kilometers from the donor tree. It is not clear what level of vector control is necessary to reduce spread of CTV. However, vector control may have potential to reduce secondary spread (Gourmet et al., 1994). Biological controls to restrict build up of citrus aphids, especially T. citricida, may be feasible (Yokomi and Tang, 1996). Although insecticides may not act quickly enough to prevent primary infection by viruliferous aphids, they could reduce local aphid populations and decrease rate of secondary spread. Insecticidal control of vector populations may have use in special situations such as in a citrus nursery or to protect Bud wood sources. A long residual systemic insecticide with minimum impact on biological control agents is preferred. CTV titer is highest when trees are forming new shoots in spring and fall. Aphid lights also peak at this time and, hence, these periods should be targeted for control actions.

2.9.8. Integrated Disease Management

Incidence and spread of CTV is a complex process that involves interaction of the plant, pathogen and vector. Conventional approaches have been directed at one or several components for CTV control. An integrated disease management (IDM) strategy should incorporate as many elements as possible based on our fundamental knowledge of the disease (Garnsey et al., 1996a).

2.10. History of Tristeza Disease in Ethiopia

The land holdings of citrus orchards surveyed varied from 0.15 ha by the smallholder farmers to 10,030 ha for Bebeka Coffee Estate farm. The area coverage of citrus plantation for these orchards ranged from a few citrus trees in the backyards of smallholders to 1,000 ha at Nura Era citrus farm. Different citrus species and varieties are cultivated in the country. The information obtained from the surveys showed that sweet orange, mandarin, lime, lemon, grapefruit, citrus hybrids, sour orange and citron were produced (Abate, 1988).

According to Mekbib (2007) report, Citrus trees that were grown by smallholder farmers were directly from seeds and were not grafted. However, citrus trees in state and private owned orchards, government nurseries and research foundation blocks were grafted. The original sources of scions and root stocks of most old orchards were unknown, but 33.3% of the respondents did not have available information. The lack of information in this regard could complicate management and breeding programs aimed at improving citrus production. However, the major sources of the recent citrus plantations included University of California at Riverside, MelkaSedi Farm in the Middle Awash, Upper Awash Agro Industry Enterprise, Research Centers, ICRISAT, and local growers. The most commonly used root stocks were Sour orange, Volkameriana and Troyer Citrange.

In Ethiopia, citrus trees have been afflicted due to different diseases incited by many fungi, bacteria, viruses and virus-like organisms. Viruses and virus-like diseases including psorosis, Tristeza and
greening were reported to be of great economic importance and thought to play a significant role in the decline of citrus plantation in the country. Canker, caused by a bacterium, is a serious disease of most commercial citrus cultivars and some citrus relatives. Based on field observations citrus producers had reported 13.6% of citrus trees die back due to virus problems and over 35% citrus attack by other plant pathogens in the major citrus farms across Ethiopia (Schubert, 1954; Mekbib, 2007).

According to Tsedeke (2008) Tristeza and greening are among the most important and widely distributed diseases of citrus in many citrus growing regions of Ethiopia. His survey result showed that there are three aphid species, viz. A. gossypii, T. aurantii, T. citricida and the citrus psyllid the only insects that occur as vectors of citrus diseases in Ethiopia. Their geographic distribution and population densities. Among the aphids recorded, A. gossypii was the least common species on citrus. It was observed at Arba Minch, Dire Dawa, Erer Gota, Melka Werer and Gibe; it was not recorded above 1250m. T. aurantii, on the other hand, was the commonest and most widely distributed aphid species in the country. It was found in large numbers at almost all altitudes covered in the survey. T. citricida, which is known to be the more efficient vector of tristeza virus was restricted to the middle and upper Awash valley, almost all of which lies below 1500m. In these areas both species of Toxoptera may occur in the same orchard or even on the same tree, but T. aurantii was by far the more dominant species.

3. Summary and Conclusion

Citrus is one of the most economically important fruit crops grown by smallholders and commercial farmers in Ethiopia. Therefore, the aim of this article is to access the production status and possible control measures of Citrus Tristeza Virus under Ethiopian condition. However, over the past 30 years export volumes have dropped due to poor quality delivered onto the market, which is mainly due to lack of improved production practices and technology transfer has hampered industry growth. In addition, citrus production and productivity in Ethiopia is seriously threatened by various diseases including citrus Tristeza which is caused by citrus Tristeza virus. Citrus is mainly propagated by grafting. Therefore, Tristeza virus is passed on from one generation to the next and also from one country to another in the absence of strict and enforced phyto sanitary and quarantine regulations. The virus is transmitted most efficiently by the brown citrus aphid. The virus Tristeza citricida is the most efficient vector and where it exists, is often the most abundant aphid on citrus. Control measure to the pathogen was Exclusion and quarantine, certification programs, vector control and integrated disease management. The virus control strategies include exclusion of the pathogen, application of planting material certification, total eradication and suppression of diseased plant, using resistant/tolerant root stock, tolerant scions, cross protection, vector control and integrated disease management are possible control methods. Therefore, the development of integrated disease management is the best of all control methods and environmentally friendly alternative practices for the management of virus and can go a long way in maintaining a sustainable environment.

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Author’s Biography

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