Current Trends in Natural Sciences Vol. 5, Issue 10, pp. 107-116, 2016

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

ARBUSCULAR MYCORRHIZAL FUNGI - AN ESSENTIAL TOOL TO SUSTAINABLE VINEYARD DEVELOPMENT: A REVIEW

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Abstract
Grapevine is one of the most important horticultural perennial crops grown in many countries from worldwide. In this paper, we review the global benefits of arbuscular mycorrhiza application for grapevine production and the impact of viticultural practices for these natural microorganisms to establish symbiotic associations with vine roots. This review aims to provide a brief overview of the status of and to outline the most important application and effects of AMF in viticulture in order to increase the sustainability of vineyards. Viticulture has to adapt to new challenges of pest and chemicals fertilizers management, climate change, global urbanization, land erosions, increasing droughts, world population growth and others factors that can affect the sustainability of viticultural production systems. One of eco-friendly approaches is to use the application of arbuscular mycorrhiza fungi (AMF). Response of grapevine to AMF application demonstrated a lot of benefits for viticultural ecosystems. Technologies on sustainable agriculture and conservation of ecosystems vineyards may consider arbuscular mycorrhiza fungi as biofertilizers. Arbuscular mycorrhiza fungi could be an effective tool for improving the agro-enviromental perfomance in viticultural farms.

Keywords: arbuscular mycorrhiza fungi, biofertilizer, plant growth and development, sustainable agriculture, symbiosis, Vitis vinifera

1. INTRODUCTION

Grapevine is one of the most important horticultural perennial crops grown in many countries from worldwide. *Vitis vinifera* and others species from *Vitaceae* family are characterized by great ecological adaptability, being planted with good agronomic results on different types of soils and climatic area from worldwide. Vitivinicultural industry is a major economic sector in agribusiness with global area under vine rose to 7.5 million hectares, 75 million tones world production of grapes, and world wine production estimated to have raised by 2.2% in 2015 than 2014 to 274 million hectoliters (OIV, 2015).

In order to achieved good grapevine yields and to ensure the demand for global viticultural products, the viticultural agro - ecosystems are subjected to a significant pressure by applying chemicals, pests, herbicides and others chemical agricultural inputs. Nevertheless, viticulture has to adapt to new challenges of pest and chemicals fertilizers management, climate change, global urbanization, land erosions, increasing droughts, world population growth and others factors that can affect the sustainability of viticultural production systems.

Avoidance of these negative influences for degradation of grapevine ecosystem services is possible by integrated new eco-friendly methods, practices and technologies. In European Union and in

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other parts of world, one of the objectives of agricultural policy is to improve the sustainability of production agro-systems by applied sustainable agricultural practices without causing damage to human health and the environment. One of eco-friendly approaches is to use the application of plant biostimulants in viticulture, and arbuscular mycorrhiza fungi (AMF) demonstrated a lot of benefits for viticultural ecosystems.

In this paper, we review the global benefits of arbuscular mycorrhiza application for grapevine production and the impact of viticultural practices for these natural microorganisms to establish symbiotic associations with vine roots. This review aims to provide a brief overview of the status of and to outline the most important application and effects of AMF in viticulture in order to increase the sustainability of vineyards.

2. GENERAL CONSIDERATION OF SYMBIOTIC RELATIONSHIP BETWEEN PLANT ROOTS AND ARBUSCULAR MYCORRHIZAL FUNGI

In the future, more sustainable horticultural practices should be developed to guarantee greater yield and yield stability, enviromental protection and food global demand. One of these practices could be represented could the arbuscular mycorrhizal fungi application used as plant biostimulants (Rouphael et al., 2015).

The term 'mycorrhiza' originates from the Greek *mycos*, meaning '*fungus*', and rhiza meaning '*root'*, and was first used in 1885 to describe the intimate association between biotrophic mycorrhizal fungi and plant roots (Garg and Chandel, 2010).

Wang and Qiu (2006) reported six categories of mycorhizas with distinct morphological characteristics: arbuscular, arbutoid, ecto-, ericoid, monoptropoid and orchid. From these mycorhizas categories, arbuscular mycorrhiza (AM) is the most common and predominant type in land plants.

Arbuscular mycorrhiza are characterized by the formation of unique structures, arbuscules and vesicles by fungi formed between roots and a particular group of fungi, which are taxonomically separated from all other true fungi belonging to the phylum *Glomeromycota* (Schübler et al., 2001).

AMF are probably the most wide spread plant symbionts and are formed by 80–90% of land plant species including grapevine (Helgason and Fitter, 2009). Almost all plant species in terrestrial ecosystems form symbioses with plant rhizosphere to take up essential nutrients including mycorrhizal fungi, nitrogen fixing bacteria and other plant-growth-promoting rhizobacteria (Parniske, 2008; Helgason and Fitter, 2009).

The AM fungal hyphae exclusive colonize the root cortex and forms intracellular structures called arbuscules which serve as the main place of nutrient exchange between the plant and the fungus (He and Nara, 2007). The fungal structures are highly branched, microscopic haustorial structures, develop extensive, below-ground extraradical hyphae, and are common to all associations of this type of mycorrhiza (Balestrini et al., 2015). Fungal penetration and establishment in the host roots involve a complex sequence of events and intracellular modifications (Bonfante - Fasolo and Perotto, 1992).

The main Arbuscular mycorrhizas are: *Glomus intraradices, Glomus mosseae, Glomus aggregatum, Glomus claroideum, Glomus clarum, Glomus etunicatum, Glomus fasciculatum, Glomus geosporum*, *Paraglosum occultum, Acaulospora*, *Gigaspora*, *Scutellospora*, and *Sclerocystis* (Jackson, 2014).

The use of some groups of microorganisms as arbuscular mycorrhizal fungi application is a good alternative to reduces chemical fertilizers are considered natural biofertilizers, since they provide the plant host with water, and nutrients, in exchange for photosynthetic products (Rouphael et al., 2015; Berruti et al., 2016; Cely et al., 2016).

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In addition to an improved nutritional supply, important aspect in crop production, most recent scientific results showed the fact that AM interactions provide other agronomical, physiological and biochemical performance of horticultural crops such as tolerance to drought and salinity conditions (Hajiboland et al., 2010; Augé et al., 2015; Boyer et al. 2015; Trouvelot et al., 2015), heavy metal contamination (Audet and Charest, 2007; Andrade and Silveria, 2008; Fernandez-Gomez et al., 2012), or disease resistance (Pozo and Azcón-Aguilar, 2007). AM fungi can also have a direct effect on the ecosystem, as they improve the soil structure and aggregation (Rillig and Mummey, 2006; Rillig et al., 2015).

In recent years, interest in AMF has focused on finding a viable method to optimize the production of AMF inoculum to use as inoculant for improving plant growth and increasing crop production (Ijdo et al., 2011; Cely et al., 2016).

AMF are sustainable tool which can enhance salt tolerance, drought tolerance, vegetative and reproductive phenological stages or other important parameters for plant growth and development (table 1).

Table 1. Different effects of arbuscular mycorrhizal fungi application on crops

Crop	Arbuscular mycorrhizal fungi	Effects/application mode/experimental conditions	Reference
Leymus chinensis	*Glomus mosseae*	-Inhanced in growth, photosynthesis and photosynthetic pigments -Improve salt-alkali stress tolerance under salt-alkali stress and nitrogen deposition	Lin et al. (2017)
Tomato (*Lycopersicon esculentum*)	*Glomus mosseae*	- Protect plants against salinity by alleviating the salt induced oxidative stress - enhanced growth, leaf area, chlorophyll content, fruit fresh weight and fruit yield of tomato plants grown under nonsaline and saline conditions -The concentrations of P and K were higher in AMF compared with non AMF plants	Latef and He (2011)
Tomato (*Lycopersicon esculentum*)	*Rhizophagus irregularis*	Increase chlorogenic acid exudation favour the growth of chlorogenate-metabolizing bacteria on the root surface or in the mycorhizosphere	Negrel et al. (2016)
Lactuca sativa	*Glomus intraradices*	Significantly promoted plant growth in lettuce under non-saline and salt stress conditions increased the stomatal conductance	Aroca et al. (2014)
Maize (*Zea mays* L.)	*Rhizophagus irregularis*	Increased Cu tolerance	Merlos et al. (2016)
Cynodon dactylon (Bermuda grass)	*Funneliformis mosseae*	-Plant biomass was significantly -Increased shoot and root Sb concentrations plant uptake in pot cultures	Wei et al. (2016)
Albizia saman Paraserianthes falcataria	*Rhizophagus clarus, Gigaspora decipiens, Scutellospora sp., Glomus clarum*	Increased shoot, P content and dry weight in a nursery and a post-opencast coal mine field	Wulandari et al. (2016)
Pepper (*Capsicum annuum* L.)	*Glomus intraradices*	Enhanced P and dry matter contents, chlorophyll concentrations (chl a, chl b and chl a + b), and amounts of some reducing sugars , sucrose and total sugar	Demir S. (2005)
Capsicum annuum L.	*Glomus intraradices*	Increased relative water content, P content, total chlorophyll, and carotenoid content; less lipid peroxidation for pepper grown under long term salt stress	Çekiç et al. (2012).

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Melia azedarach L.	*Glomus geosporum*	-Significant enhanced plant height, stem girth, plant biomass and plant N, P, Cu, and Zn contents - Vigorous and healthy seedlings	Rajeshkumar et al. (2009)
Mentha arvensis L.	*Glomus aggregatum, Glomus mosseae, Glomus fascicula-tum, Glomus intraradices*	Improves growth, yield and quality potential biofertilizers in salt-stressed soils	Bharti et al. (2016)
Durum wheat (*Triticum durum* L.)	*Glomus mosseae*	Increased soil N mineralization rates and total plant N uptake	Saia et al. (2014)
Petunia hybrid, Callistephus chinensis, Impatiens balsamina	*G. gigaspora spp. Scutellospora spp.*	- AMF improve both vegetative (dry matter) and reproductive (number of flowers) phenological stages - Significant increase in P and K uptake in shoots of all the three ornamentals in greenhouse conditions	Gaur and Adholeya (2000)
Dianthus caryophyllus	*Glomus intraradices*	Increased of N, P, and Ca and the reduction of toxic ions (Na and Cl) in leaves, stems, flowers, and roots	Navarro et al. (2012)
Fragaria x ananassa	*Glomus caledonius, Glomus mosseae, Rhizophagus irregularis*	- Enhanced plant growth and improved salt tolerance by increasing the proportion of medium ($0.5<\phi\leq1.5$ mm) and coarse ($\phi>1.5$ mm) diameter roots under salt conditions in greenhouse - Cultivars respond differently to fungal inoculants	Sinclair et al. (2014)
Cucumis melo	*Glomus mosseae, Glomus versiforme, Glomus intraradices*	-Higher tolerance to drought enhanced antioxidant activities, soluble sugars contents, net phostosynthetic rate and photosynthetic water use efficiency -Enhanced plant height, root length, stem diameter, fresh weight, dry weight and ratio of root/shoot in well – watered and water deficit in greenhouse conditions	Huang et al. (2011)
Acacia auriculiformis	*Glomus fasciculatum Glomus macrocarpum*	-Improve root colonization and spore production -Significantly higher root and shoot weights -Greater nutrient acquisition -Increase salt stress tolerance	Giri et al. (2003)
Apple (*Malus domestica*)	*Glomus versiforme, Claroideoglomus etunicatum Rhizophagus intraradices*	-Enhanced growth parameters and nutrient uptake of three apple rootstocks (M.9, M.7 and MM.106) -AMF inoculation significantly affected leaf concentrations of N, P, Ca, Mg, Zn, and Fe in apple rootstocks - *G. versiforme* had the highest impact for growth parameters	Hosseini and Gharaghani (2015)

3. THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON GRAPEVINE - A KEY TO SUSTAINABLE VINEYARD DEVELOPMENT

Arbuscular mycorrhizal fungi are important partners for plant and this fungal community normal in field conditions is presented in grapevine soils and colonised the root system of grapevine (Cheng and Baumgartner, 2004; Balestrini et al., 2010) and especially Glomus aggregatum is a fungus frequent found in arid land areas (Karagiannidis and Nikolaou, 1999; Schreiner, 2003; Uhlmann et al., 2006).

Arbuscular mycorrhizal fungal (AMF) biodiversity in vineyards were studied to identified the factors that can affect the intensity of root system colonization with these natural beneficial organisms (Schreiner and Mihara, 2009; Balestrini et al., 2010; Likar et al., 2013). Balestrini et al.

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ISSN: 2284-953X
ISSN-L: 2284-9521

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(2010) observed that AMF community seems to be affected by soil characteristics. Likar et al. (2013) stated that arbuscular mycorrhizal are influenced by environmental factors, while many scientists reported that agronomic practices influenced the mycorrhizal colonisation of grapevine root system. Some agronomic practice had a negative impact on mycorrhizal colonization. Karagiannidis et al. (2007) found urea suppressed AM fungal root colonization and sporulation.

Soil salinity is an important problem present mainly in arid, semi-arid and other zones from worldwide that reduce agricultural productivity. It is one of major abiotic factors limiting viticultural production. In order to alleviate the negative impact of soil salinity, many studies have focused on grapevine performance under salt abiotic stress conditions using the potential of AM fungal community to improve the salinity tolerance.

Khalil (2013) reported that Dogridge (*Vitis champini*), 1103 Paulsen (*Vitis berlandieri* x *Vitis rupestris*), and Harmony (*Vitis champini*) one – year grapevine rootstocks, inoculated with Glomus intraradices were irrigated with three different NaCl concentrations. The results indicate positive effect of AMF inoculated plants for growth parameters (plant height, stem diameter, leaf area, shoot and root biomass) compared to the non-inoculated plants in salt conditions. The higher crop performance in inoculated grapevine with AMF was attributed to a lower concentration of Na and Cl and the higher K, Mg concentration in leaf tissue and also to the higher K/Na ratio (Khalil, 2013). These results demonstrate the beneficial role of AM symbiosis in improving grapevine salt tolerance (Belew et al. 2010; Khalil, 2013). Belew et al. (2010) documented the effect of soil salinity in the presense of mycorrhizal fungus (*Glomus fasciculatum*) on grapevine rootstock, namely Dogridge (*V. champini*), Salt Creek (*V. champini*), St. George (*V. rupestris*) and 1613 (*V. riparia* x *V. rupestris* x *V. vinifera* x *V. candicans* x *V. labruska*). The cuttings were inoculated with *Glomus fasciculatum* in the nursery using 5 g of inoculum per cutting and the main measurements were shoot length, internode length, leaf number, total dry weight, spore count and root colonisation of grape rootstocks. Total dry weight decreased with increasing salinity levels and inoculation of the rootstocks with *Glomus fasciculatum* increased plant growth and dry matter accumulation. However, this experiment showed that grapevine rootstocks have different reaction and agronomic performance under salt conditions.

Camprubi et al. (2008) investigated the response of the grapevine rootstock Richter 110 to inoculation with native and selected arbuscular mycorrhizal fungi and growth performance Cabernet Sauvignon grafted on Richter 110 rootstock. Authors indicated that only selected *Glomus intraradices* BEG 72 was able to improve plant development. Plant growth response (fresh weight, dry weight, number of leaves per plant) and percentage of AM colonization in Richter 110 rootstock was more positive for plants inoculated with *Glomus intraradices* BEG 72 after six months growth under greenhouse conditions compared with native AM fungal isolates. For instance AM root colonization was 60% for plants inoculated with *Glomus intraradices* BEG 72 and from 33% to 54% for plants inoculated with native AM fungal.

In order to improve the nursery performance, the response of root system to mycorrhizal fungi was tested in some experiments. Root morphology for three grapevine rootstocks were analyzed in nursery production under the influence of AM inoculation. The rootstock used, Richter 110, has been shown to respond to the AM inoculation with a significant growth increase in the first and second order lateral roots, compared to other rootstocks, where changes in root architecture were not as important (Aguín et al., 2004). This study concluded that AM inoculation in the rooting media could be a useful tool for improving the grapevine performance in the nursery production. Also, Cheng and Baumgartner (2004) found that mycorrhizal colonization and root biomass of grapevine nursery was significantly higher for the experiment with AM inoculation.

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Differences in root colonization by AM fungi have been observed in grapevine rootstocks. Karagiannidis et al. (1995) and Linderman and Davis (2001) found relatively small differences in colonization among Kober 5BB, Richter 110, and 41B or among Riparia Gloire, Kober 5BB, SO-4, 420A, and 101-14 Mgt rootstocks. Karagiannidis et al. (1997) found significant differences in the level of root colonization among Richter 110, Ruggeri 140, 1103 Paulsen, and 41B rootstocks examined after eight years in a field experiment. Grafted cu1tivars on these grapevine rootstocks were found to have some influence on the degree of AM root colonization and on the population of spores, but had no effect on the formation of vesicles and arbuscules. The scientist showed that the number of vesicles varied between 16 and 47 and the number of arbuscules between 5 and 26 per cm of infected root. Spore number produced by the mycorrhizal fungi in the rhizosphere ranged from 196 to 280 per 100 g of soil.

Vine nutrition is essential for quality of grapes in order to achieve positive results in vineyard management. Most of vineyard soils are less fertile than soils for other crops. Many studies have reported that AMF promoting nutrient uptake, especially increase phosphor supply. For this reason AM symbiosis can reduce the need of fertilizers and could be an alternative to chemical fertilizers for providing the plant nutrient requirement.

The grapevine rootstock grafted on Pinot noir clone FPMS 2A were evaluated under natural field conditions for root colonization by AM fungi (Schreiner, 2003). Kober 5BB, SO-4, and Ruggeri 140 had the greatest levels of AM colonization and produced the most vigorous scions over the three years of experiment in natural field conditions. Schreiner (2003) demonstrated that small differences in the capacity to form mycorrhizas exist among grapevine rootstocks, but other factors, including crop load and soil moisture, have a major influence on AM root colonization.

The influence of native and nonnative arbuscular mycorrhizal fungi (G*lomus mosseae*, *Glomus intraradices*, and *Scutellospora calospora*) on growth plant and nutrient acquisition by Pinot noir grapevine cuttings was studied in soils with different available P by analyzing the response to shoot length, dry biomass of whole shoots and root, root length, AMF colonization (% root length), and whole plant nutrient concentrations and contents at harvest. The conclusions showed that grapevine grown in soils with low available P need good root colonization with AM fungi to better supply P and the vines grown in more fertile soils are less dependent by the presence of mycorrhizal fungi on grapevine rhizosphere (Schreiner, 2007).

Caglar and Bayram (2006) tested the impact of *Glomus etunicatum*, *Glomus caledonium*, *Glomus darum*, *Glomus mosseae*, and mixed inoculum on the nutritional status of one-year-old grapevine rootstocks cuttings (420 A, 41 B, 1103 P, and '*Rupestris du Lot*'). This impact was demonstrated by the determinanted the following measurements: leaf area, N, P, K, and total sucrose contents. Inoculations with G. etunicatum and G. darum was able to significantly eneganced the leaf areas of 41 B, 420 A, and 1103 P rootstocks. Caglar and Bayram (2006) concluded that AMF inoculation increased leaf P concentration, but not N and K concentrations.

Eighteen year old vines of cv. Perlette were inoculated with three strains of AM namely *Glomus mossae*, *Glomus deserticola* and *Gigaspora calospora* in order to evaluate the influence of AMF inoculation on growth plant, nutrient uptake and plant productivity in terms of grape yield and cane productivity (Usha et al., 2005). The results indicate that AMF infections improved nutrient uptake, increased yeield, and hastening of bud sprouting, flowering, berry set and ripening. *Glomus deserticola* play an important role for nutrient uptake and this aspect can be included in the fertilizer management of vineyard as an alternative to chemical fertilizers.

Three species of arbuscular mycorrhizal fungi, namely *Glomus mosseae*, *G. fasciculatum*, *G. intraradices* were tested to investigate the effect of AMF on *in vitro* and *ex vitro* performance of four table grapevine explants (Eftekhari et al., 2012). Eftekhari et al. (2012) reported that AMF

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ISSN-L: 2284-9521
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inoculums in the protocol of *in vitro* technology increased total phenols, sugar content, chrolophyll content, root colonisation and root length.
Cangahuala-Inocente et al. (2011) reported that SO4 rootstock (*Vitis berlandieri* x *Vitis riparia*) 30-day old cuttings were colonized with *Glomus irregulare* and *Glomus mosseae* and compared with non-colonized vines. They stated that frequency of infection in the root system was similar for the two AMF inoculation with *Glomus irregulare* and *Glomus mosseae*, while abundance of arbuscules were significantly higher ($p<0.05$) in grapevine colonized with *Glomus irregulare* than in plants colonized with *Glomus mosseae*. Effect of mycorrhizal inoculation treatment on shoot biomass and root biomass after 5 weeks of inoculation with *Glomus irregulare* or *Glomus mosseae* indicated that the values of these parameters were higher in AMF colonized grapevine.
Irigation is an important viticultural practice for fruit quality and grapevine yield. This practice increase vineyards costs and water stress for grapevine is a major problem for the performance of viticulture ecosystems. Schreiner et al. (2007) demonstrated that total AMF may play a significant role in vine response to water stress and arbuscular colonization of grapevine fine roots by mycorrhizal fungi increased as soil water content decreased.
Karagiannidis et al. (2007) indicated that AMF was able to produce higher shoot dry weight, number of leaves, and higher content of P and K in leaves than non-mycorrhizal plants. In the same time the concentration of microelements Zn, Mn, Fe, and Cu in leaves was higher in non mycorrhizal plants than mycorrhizal. Regarding the quality of grapes, Karagiannidis et al. (2007) found no difference amomg mycorrhizal and non-mycorrhizal plants in berry total acidity and soluble solids.
Nicolás et al. (2015) indicated that AMF improved the grapevine water status, photosynthesis, and promoted better uptake of phosphorus, potassium and calcium. Authors conclude that AMF application technique can be recommended for sustainable agriculture in arid and semi-arid areas and suggested that periodic monitoring of the percentage of mycorrhizal colonization and re-inoculations with AMF is necessary in order to achieve positive effects for grapevine growth and development.
Potential protection of AM fungi against biotic stresses has been described mainly against root pathogens and several studies showed that AM symbiosis can enhance grapevine tolerance to fungal pathogens and reduce nematode development (Petit and Gubler, 2006; Li et al., 2006; Nogales et al., 2009; Hao et al. 2012).

4. CONCLUSIONS

Arbuscular mycorrhizas is a group of soil biota that can contribute to crops performance. Arbuscular mycorrhizal fungi are very common in terrestrial ecosystems and their importance for agricultural systems has been widely recognized by numerous scientific achievements. These resources improve plant plant productivity and quality for many species and could be a good strategy to include as biofertilizers in conventional or oraganic agricultural systems. Arbuscular mycorrhizal fungi in many studie enhanced agronomic performance for many horticultural species. Response of grapevine to AMF application demonstrated a lot of benefits for viticultural ecosystems, among which: protecting against root pathogens, enhance plant growth and development, improve nutrient acquisitions, alleviate grapevine water stress, improve tolerance to salinity stress conditions, etc. Recent findings demonstrated that arbuscular mycorrhizal fungi are an alternative sustainable for use of chemical fertilizers. Colonisation of grapevine root system with arbuscular mycorrhizal fungi is influenced by soil features, environmental factors and viticultural practices. The beneficial effects of arbuscular mycorrhizal fungi on grapevine are essential for the sustainable management of viticultural ecosystems.

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ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

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ISSN-L: 2284-9521

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ISSN: 2284-9521
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