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EVALUATING THE ABSORPTION RATE OF MACRO AND MICROELEMENTS IN THE LEAF OF GRAPE *Sefid Bidaneh cv.* UNDER DROUGHT CONDITIONS

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ABSTRACT

In order to determine the effects of root inoculation of grape *cv. Sefid bidaneh* with three species of mycorrhiza fungus namely *G. fasiculatum*, *G. intraradices* and *G. mosseae* on the absorption of macro and microelements including phosphorus, potassium, and magnesium in leaf under drought conditions, an experiment was designed in a factorial based on randomized complete block design with four replications. The results showed that an increase in drought stress resulted in the reduction in macro and micronutrients absorption, and inoculation with mycorrhiza fungus could have a positive effect on the greater absorption of these elements compared to control. The amount of leaf phosphorus in the treatment of *G. fasiculatum* was the highest so that it showed a significant difference from *G. mosseae* and control treatments. The highest amount of potassium was found in *G. mosseae* treatment that showed a significant difference from other treatments. Based on the results, *G. fasiculatum* treatment had the highest content of magnesium, while the lowest rate was obtained in *G. intraradices* treatment.

1. INTRODUCTION

Grape, the main horticultural crops of Iran, has the biggest cultivation area and has economic value followed pistachio and date [1]. The work of [2] considered near east region as the center of origin for grape according to the studies on plant geography and archeology. Based on plant archeological studies, the grape was first domesticated in the second half of fourth millennium BC in two adjacent regions of Mezopotamia (including south of Anatoli, Syria, North of Lebanon, Kurdistan, and west of Iran) and south of Caspian Sea.

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2. BIDANEH GRAPE

This cultivar is also called Keshmeshi and distributed in most grape cultivation regions of Iran. Bidaneh grape is white and red in color, both of which are considered as the best table grapes. The cultivation area of white grapes is higher than the red one. Bidaneh grape is fast-growing variety and the length of its shoots reaches 2.5 m. Terminal bud of this variety is closed, fuzzy and white in color. Its leaves are dark green in color and generally have no fuzz, and their veins are bright yellow. Nodes are green and turn to purple in the bottom part. This variety has green vigorous tendril and perfect flowers (hermaphrodite). The cluster is relatively long and has medium density and green pedicle. Berries are seedless, have a round shape, and the thickness of their skin is medium. This variety sees its fruits ripen in December.

2.1 DROUGHT STRESS

Water scarcity is one of the most important factors limiting the yield of fruit trees in arid and semi-arid regions. Evaluating the yield of fruit trees and using soil beneficial microorganisms as bio fertilizers in order to reduce the damages resulting from environmental stresses are novel solutions in sustainable agriculture in arid and semi-arid regions [3-4]. Environmental conditions of either air or soil which prevent plant access to adequate water for performing vital functions, and the continuation of these conditions which result in the loss of plant tissues water, is called drought [5]. When the plant is dehydrated that plant cells lose their swelling status and transpiration rate is higher than absorption [6]. Along with the reduction of water in the soil and lack of its replacement, water potential in the rhizosphere and subsequently in the plant is reduced. Under these conditions, if the intensity of water stress is high, it leads to a sharp reduction in photosynthesis, intervention in physiological processes, yield reduction and finally plants drying. The main cause of water stress in plants is transpiration and inadequate water absorption, or a combination of these two factors. Factors such as leaves structure and area, size of stomata pores, number of pores and other important factors in water vapor gradient between plant and atmosphere control the transpiration [7]. The reaction of the plant to water scarcity is determined by plant species, duration of drought stress, age and development stage of the plant, plant cell and organ type, sub-cellular components, and plant structure.

2.2 MYCORRHIZA FUNGI

The term Mycorrhiza indicates the symbiotic association between fungus and plant roots. The formation of mycorrhiza is the result of fungal penetration into inter-cellular spaces of root bark, where they form a network of fungal mycelium and a woven mesh around root that extends well in the soil and facilitates water absorption.

The term mycorrhiza consists of two words: Myco means fungus and Rhiza means root, which shows the relationship between fungus and host's plant root. In this system, fungus develops an intensive network of thread-like hyphae called mycelium around the host plant's root. In general, the development of the mycorrhizal system can be seen in the majority of plants which include 83% of dicotyledons and 79% of monocots.

Establishment of arbuscular mycorrhiza (AM) symbiosis represents an important innovation for plant tolerance and may be considered as the first evolutionary mechanism against the conditions of low phosphorus in natural ecosystems. Therefore, arbuscular mycorrhizal fungi play an important role in the cultivation of plants in dry regions, which in turns result in the evolution of drought-tolerant plants. Various researches showed that mycorrhizal system absorbs phosphorus, nitrogen, potassium, zinc, copper, sulfur, magnesium, manganese, calcium and iron, and transfers them to plant. In general, absorption mechanism is through the increase of available soil volume by fungal hyphae, which penetrate in soil pores that is not possible for root hairs to penetrate (diameter of root hair is at least 20 micrometer, while the maximum diameter of hyphae is 1 to 2 micrometer). Furthermore, hyphae increase the absorption of nutrients through the increased surface area or increasing the effective length of the root. Each cube centimeter of soil contains 2-4 cm root, 1-2 m root hair and over 50 m hyphae [8].

2.3 MYCORRHIZA FUNGUS IN GRAPE

Owing to having genes tolerant to drought and soil lime, grape *var vinifera* nowadays is cultivated as rainfed in most regions with enough rain [9]. However, harsh drought stresses in some years and in sensitive phonological stages like fruit set reduce significantly the yield. On the other hand, cultivation of one-year seedlings in these dry regions encounters problems due to low water amount and unfavorable soil conditions. Nowadays, besides using tolerant and precocious varieties, tolerant rootstocks and water management (collection of rainwater, regulated deficit irrigation, and partial root-zone drying), inoculation of grape roots with arbuscular mycorrhizal fungi (AMF) are also used for the development of orchards in arid and semi-arid regions [10].

Mycorrhiza is a fungus which is found almost in all soils and establishes a connection with the roots of the plant. In the symbiosis of this fungus with grape root, nutrients absorption is increased by fungus, and, on the contrary, vine provides the fungus with assimilates. This kind of relationship is called symbiosis, and both vine and fungus are symbionts. Nutrients exchange inside grape root cells occurs through branch-like structures or arbuscular, which increases the surface area between the host and fungus. The thinnest root of the vine is 500 to 1000 times thicker than mycorrhiza hyphae. Various pot and field studies showed that grape seedlings containing mycorrhiza under rainfed conditions and in unfavorable soils had better growth and were able to absorb the greater level of water and elements such phosphorus and iron compared to control. Furthermore, they showed higher resistance to soil-borne pathogens. It was also proved that mycorrhiza resulted in the maintenance and stability of soil texture by attaching soil particles by hyphae [11].

In general, the role of mycorrhiza can be stated in 5 parts: 1. Increasing the nutrients absorption, 2. Protecting against soil-borne pathogens, 3. Maintaining and stabilizing soil texture through gluing soil particles by hyphae network, 4. Increasing the tolerance to drought through greater absorption of water and phosphorus, 5. Increasing soil fertility [11].

Different studies showed that water stress caused a limitation in nutrients absorption by the plant, which might be due to transpiration reduction, disruption to active transport systems, membrane permeability, and thus reduction of root absorbability. Due to the slow rate of plant growth under drought conditions, dilution phenomenon of nutrients does not occur in the plant. As one of the most important factors limiting the production of agricultural crops in the world, drought causes annually severe damage to agricultural products. The average rainfall of Iran is less than the global average [12]. Approximately 65% of Iran's area is arid and semi-arid regions with the mean rainfall less than 150 ml per year. Drought stress in grape leads to drying of petioles, tendrils, and young leaves. In addition, long drought stress may result in the creation of necrotic spots in the margin of grape leaves, in particular, bottom leaves. Leaf color is also an indicator showing the effect of drought stress, so that young leaves at the end of shoots have yellowy-green color and adult leaves have grey-green color. With the continuation of drought, early death occurs in bottom leaves. During harsh drought stress, the volume of shoot and leave reduces and thus the rate of transpiration decreases [13]. Numerous studies showed that phosphorus, nitrogen, potassium, zinc, copper, sulfur, magnesium, manganese, calcium and iron were absorbed by the mycorrhizal system and were transferred to plant. Phosphorus ion is absorbed strongly by soil clay under drought conditions, and an only a small part of it is in a solution state. Under drought conditions, absorption of phosphorus ion reduces not only because of its low solubility but also because of reduced absorbability of the roots [14]. Generally, the absorption mechanism is through the enhancement of available soil volume by fungus mycelium. Among the nutrients, phosphorus absorption is highly affected by mycorrhiza. When soil phosphorus is low, the mycorrhizal system increases phosphorus absorption and therefore plant growth. A big part of available phosphorus in soil is insoluble and unusable for the plant.

During a study about the effect of mycorrhiza fungus on nutrients absorption by grape root in poor soils [15] reported that the highest effect of fungus was seen on phosphorus absorption. Furthermore, [15] noted that due to the increase in vegetative growth of seedlings containing mycorrhiza, dilution of these elements in the leaf is possible. Mycorrhizal symbiotic relationship plays a major role in decomposition of soil organic matter, mineralization of nutrients and nutrients cycle [16]. The increase in water and nutrients absorption by mycorrhiza can be due to the growth of fungal hyphae up to 20 ml from root area in comparison with 1.5 ml growth of hairy roots as well as low penetration of root compared to the high penetration of hyphae into soil pores. Hyphae of these fungi penetrate into the sections of soils which is not possible for root to penetrate, therefore increase the exchange rate of nutrients and water with soil soluble components [17].

Improvement of mycorrhizal crop production under drought stress is associated with high concentrations of immobile nutrients such as phosphorus, zinc, and copper [17]. reported that enhancement of dry matter of underground and aerial organs in the treatment of mycorrhizal inoculation is probably owing to increase in water and nutrients concentration, better transport of these materials to aerial organs and increase of plant photosynthesis.

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL MATERIALS, DESIGN TYPE, AND EXPERIMENTAL TREATMENTS

In the present study, the effect of roots inoculation of one-year-old grape with some species of mycorrhizal fungus on water and nutrients relations was investigated. The experiment was conducted in factorial based on a randomized complete block design with two factors and four replications in two consecutive years (2013-2014). The first factor was inoculation with three mycorrhiza fungus species namely G. mosseae, G. fasciculatum and G. intraradices along with control (no inoculation), and the second factor was three levels of irrigation (35, 55 and 75% of field capacity water). The soil of pots contains half sand and half field soil. First, cuttings of grape cv. *sefid Bidaneh* were prepared in the required number and were rooted in a sand medium based to Mama of the method. In the same medium, half of the plants were inoculated with a suspension of arbuscular mycorrhizal fungus, and the rest of the plants were considered as control.

3.2 PREPARATION OF MYCORRHIZAL PLANTS

An inoculum of mycorrhiza fungi (spore, mycelium, mycorrhizal roots, and soil) was supplied from Turan Biotech Company, Shahrood, and was reproduced on sorghum root. For producing mycorrhizal grape seedlings, hardwood cuttings were placed in rooting medium that was mixed with mycorrhizal fungi inoculum with the ratio of 15 ppm. In order to be ensured that root is colonized, sampling from the root was done weekly. The percentage of root colonization was determined after staining the roots with Trypan 05%. In the next step (at the end of winter), rooted inoculated seedlings were transferred to 20-liter plastic pots. Two-bud pruning was done on grape seedlings in the early spring. After 20-cm growth of seedlings, drought stress was applied as follows. Plant available water was obtained based on weight percentages of field capacity and wilting point, and was expressed as weight (W) by considering the weight of soil in each pot; therefore available water was obtained and stress treatments were applied. Irrigation treatments were 35, 55 and 75% of field capacity, and irrigation schedule was performed at 2-, 4- and 6-day intervals.

3.3 MACRO AND MICROELEMENTS IN ROOT AND LEAF

The amounts of nitrogen, phosphorus, potassium, magnesium, iron, and zinc in leaves, as well as nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc and manganese in roots, were measured [35]. Leaf samples were selected randomly in the final stages of growth for measuring growth parameters. The Samples were first washed and dried in an oven (72 ° C for 48 hours), then powdered by using the mill, and finally, the extracts were prepared based on digestion by the dry burning method. Phosphorus content was measured by the colorimetric method (yellow color of molybdate vanadate) and by using spectrophotometer at the wavelength of 470 nm, and finally, the amount of phosphorus was calculated using equation (1).

$$P = \text{concentration from regression graph} \times (10) \text{ given dilution} \times 1.33 \times 100/3\text{g sample} \times 10000 \quad (1)$$

Potassium content was determined by using flame emission method and using Flame Photometer device (Jenway PFP10, England), therefore, standard solutions of zero, 20, 30, 40 and 50 mg/l potassium chloride (KCl) were first prepared and their standard curves were drawn by using the Flame Photometer. Then, 10 ml of the plant extract was brought up to the volume of 100 ml with distilled water, and a potassium concentration of the sample was read by Flame Photometer and expressed in mg per liter. Finally, using equation 6-3, potassium content was estimated in milligram per 100 grams of dry leaf. Furthermore, for measuring nitrogen, one gram of ground sample from each treatment was weighted and then the amount of nitrogen was measured by using Kjeldahl method,

$$(a-b)\times\frac{1}{1000}\times\frac{V}{W}\times\frac{100}{D.M} \quad (2)$$

where a- Potassium concentration in the diluted sample (milligram per liter),

b- Potassium concentration in control (milligram per liter),

V- Extract volume obtained from digestion operation (ml),

W- Plant sample weight (gram),

D.M- dry matter (percent)

3.4 STATISTICAL ANALYSIS OF THE DADA

Before performing data analysis, the normality of the data was performed using the Kolmogorov-Smirnov Test (K-S) in SPSS® software. To analyze the data, SAS software was used. The differences among treatments means were compared using LSD.

4. RESULTS AND DISCUSSION

4.1 ANALYSIS OF VARIANCE RELATED TO THE EFFECTS OF MYCORRHIZAL FUNGI AND WATER STRESS TREATMENTS ON THE AMOUNT OF MACRO AND MICRONUTRIENTS IN LEAVES

Consider the effects of mycorrhizal fungi and water stress treatments, the analysis of variance on the amount of macro and micronutrients in leaves is given in Table 1.

Table1: Analysis of variance of macro and micronutrients levels in leaves

source of variation	Degree of freedom	mean square					
		N (%)	K (%)	P (%)	Mg (%)	I (ppm)	Zn (ppm)
Fungus	3	0.188 ^{ns}	0.073 *	3.333 **	0.084**	2682.027^{ns}	13.913 ^{ns}
Irrigation	2	0.127 ^{ns}	0.085 *	0.928 ^{ns}	0.004^{ns}	1294.719^{ns}	5.118 ^{ns}
Fungus × Irrigation	6	.0154 ^{ns}	0.038^{ns}	0.368^{ns}	0.011^{ns}	3449.719^{ns}	8.209 ^{ns}
error	36	0.077	0.018	0.693	0.009	2430.119	9.758
Coefficient variation		12	10	15	9	11	8

ns means lack of significant difference, ** and * represent significant differences at 1% and 5%, respectively.

4.2 LEAF POTASSIUM

Analysis of variance showed that the effects of fungal and irrigation treatments on leaf potassium content were significant at 5%. The amount of potassium in leaves of vines inoculated with the fungus G. mosseae was the highest and showed a significant difference from the potassium rate found in other fungi and control treatments (Figure 1).

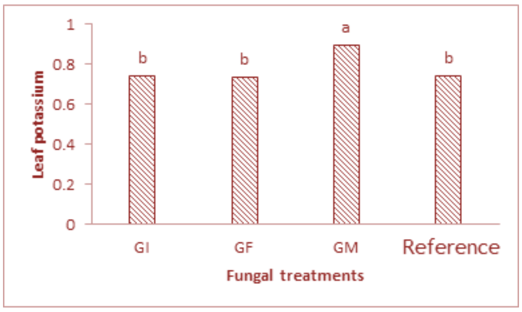

Figure 1: Means comparison of potassium content in leaf
GM: Glomus mosseae, GF: Glomus fasciculatum, GM: Glomus intraradices

The increase of potassium in leaves can be associated with the absorption of the element from the root and its transfer to leaves in order to increase osmosis potential and maintain cell turgor. The element also plays a role in the increase of stomatal conductance [18]. The results reported by [19] showed that potassium and calcium levels in leaves of seedlings inoculated by arbuscular mycorrhizal fungus were higher than those found in leaves of seedlings without fungus inoculation, however, the differences were statistically significant only under water stress conditions. [20] found that inoculation of maize plants with mycorrhizal fungi under water stress brought about a significant increase in the amount of seeds potassium. Arbuscular mycorrhizal fungal colonization improves osmotic adjustment on the basis of enhancement of potassium and calcium concentrations, thus leading to an increase of drought tolerance [21].

In a study about the effect of G. inratadices and G. mosseae inoculants, solely or in combination, [22] found that using mycorrhizal fungi increased potassium content in leaf, and the highest effect was due to G. mosseae fungus. Our results are consistent with the findings of this study.

Report [23] indicated that inoculation of maize with *G. mosseae* and *G. intraradices* had a significant effect on the potassium content of the plant. [24] reported potassium uptake in *Lavandula stoechas* L. as a result of mycorrhizal inoculation. In the current study, a mycorrhizal fungus of *G. mosseae* had the same effect reported in studies carried out by other researchers.

The means comparison of this study showed that the highest amount of potassium was observed in the stress level of FC 75% (Figure 2).

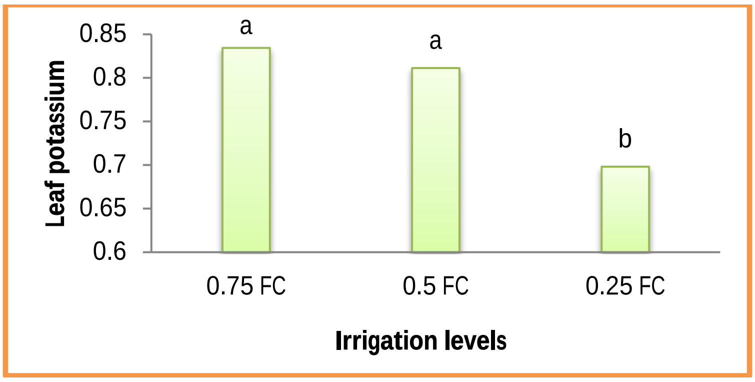

Figure 2: Means comparison of leaf potassium under different levels of irrigation.

Levels of potassium and calcium in *Poncirus trifoliata* treated with mycorrhizal fungus were higher than those in plants without fungi inoculation [25]. [26] found that the amount of potassium in seeds significantly increased in maize inoculated with mycorrhizal fungi under water stress. Increase in potassium and calcium concentrations is the result of osmotic adjustment improvement by arbuscular mycorrhizal fungal colonization, which causes an increase in drought tolerance [21].

4.3 LEAF PHOSPHORUS

Analysis of variance showed that fungal and water treatments had a significant effect on the amount of phosphorus at 1%. Phosphorus content in vine leaves inoculated with *G. fasiculatum* and *G. intraradices* fungi were higher than *G. mosseae* and control treatments. In other words, these fungi had high efficiency in absorption and transportation (Figure 3).

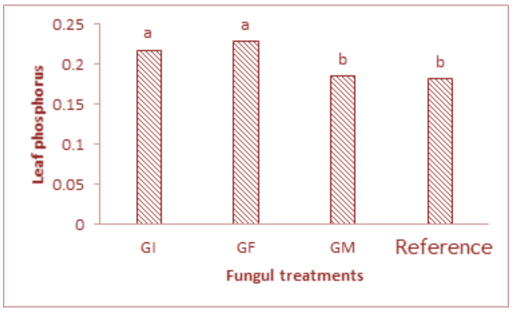

Figure 3: Means comparison of phosphorus rate in the leaves affected by fungal treatments.

Considering the low mobility of phosphorus in the soil which shows a further reduction in the time of drought stress, mycorrhizal fungi are able to increase absorption area of root and nutrients transport to root by the development of external hyphae and morphological changes of root [27].

In order to evaluate the effects of mycorrhizal fungus and deficit irrigation, [28] inoculated 8 rootstocks of vine with the mycorrhizal fungus and cultivated the grapes in sandy soils poor in phosphorus and organic matter. Their results showed that the leaf phosphorus rates in plants inoculated with mycorrhiza fungus were higher than control.

The investigation [15] about the effect of mycorrhizal fungus on the absorption of mineral nutrition by grape root in poor soils, reported that besides the highest effect of fungus on phosphorus absorption rate, other nutrients including zinc, nitrogen, potassium, calcium, and iron are also effected. Also, [15] reported that by increasing the vegetative growth of mycorrhiza-inoculated seedlings, the possibility of concentration dilution of these elements in the leaves exists.

Studying the field response of mung bean inoculated with two species of mycorrhizal arbuscular, [29] reported that the rate of roots infection in mung bean treated with *G. intraradices* was higher when compared with *G. mosseae*, and colonization percentage of *G.intraradices* relative to *G. mosseae* decreased to lesser rate by increasing water stress.

By increasing water stress level, the percentage of root colonization in *Poncirus trifoliata* rootstock greatly reduced; colonization percentage in treatments of mycorrhizal fungi inoculation was between 34 and 81%. Root colonization percentage of *G. mosseae* species was higher compared with *G. versiforme* in all irrigation intervals [30]. The results of this study were similar to the results of [25] on citrus under water stress conditions. Root colonization percentage in *Lavandula stoechas* L. inoculated with *G. mosseae* and *G. intraradices* increased as much as 35 to 100%. Figure 3 shows the positive effect of fungi on phosphorus absorption, which is in agreement with the studies of other researchers.

4.4 LEAF MAGNESIUM

Based on analysis of variance, water, and fungal treatments showed a significant effect on the amount of magnesium in leaf at 1%. The average magnesium content in *G. fasiculatum* treatment was the highest, while *G. intraradices* showed the lowest amount of leaf magnesium. Control treatment showed no significant difference from *G. fasiculatum*, so did *G. intraradices* from *G. mosseae* (Figure 4).

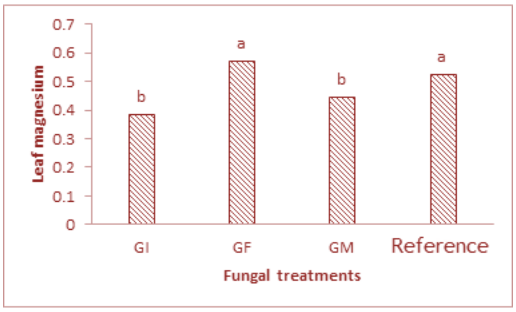

Figure 4: Means comparison of magnesium content in leaves affected by fungal treatments.

Researchers related the increase in the absorption of micronutrients to lower redox potential in the rhizosphere, increase of chelating secretion and higher reduction of pH [31] in the rhizosphere of plants inoculated with fungus compared with plants without fungus inoculation. Greenhouse cultures and field experiments have shown that the effect of mycorrhizal fungi on the uptake of micronutrients is higher than macronutrients [32]. The effect of mycorrhizal fungus represents the efficiency of these fungi in rootstocks in the symbiotic system. In this system, environmental conditions play a vital role in the effectiveness of fungus [33]. Water deficit results in the reduction of root branches and damages to root form, therefore absorption of nutrients by root and final yield decrease. Mycorrhizal fungi due to having hyphae with a smaller diameter than roots penetrate into tiny pores of soil and absorb moisture and nutrient elements of the soil [34]. [26] found that maize plants inoculated with mycorrhizal fungi under water stress showed an increased amount of nitrogen, potassium, manganese, magnesium, and zinc. In the present study, an increase was found in the content of magnesium, zinc, and potassium, which is in line with the results reported by [26].

5. CONCLUSION

In general, fertilizers containing organic materials such as vermicompost satisfy the nutritional needs of plants and increase the amount of biomass. In this research, the effect of mycorrhiza fungi on the absorption rate of macro and microelements in the leaf of grape cv. sefid bidaneh under drought conditions were investigated. By increasing water stress level, the percentage of root colonization in *Poncirus trifoliata* rootstock greatly reduced; colonization percentage in treatments of mycorrhizal fungi inoculation was between 34 and 81%. Results of this study confirmed that Mycorrhizal fungi to be strong growth stimulants in grape plantlets by optimizing P absorption and ensuring a greater supply of macronutrients and micronutrients.

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