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INFLUENCE OF *ARBUSCULAR* MYCORRHIZAL FUNGI, RHIZOBIUM INOCULATION AND ROCK PHOSPHATE ON GROWTH AND QUALITY OF LENTIL

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Abstract

Effective inoculation of legumes has the ability not only to ensure nutrients availability to plants particularly in N & P-limiting (due to improvement in nutrients fixation) environments but also can manipulate the environmental hazards associated with over inorganic fertilization. To support this view, the current experiment was conducted to study the influence of rock phosphorus fertilization, Arbuscular Mycorrhizae (AM) and Rhizobium inoculation on growth and yield parameters of *Lens culinaris* (NARC.2008-4). In addition, the current experiments aimed to evaluate the effect of different inoculation practices on crop quality in comparison with control (no inoculation).The experiment was laid out in randomized complete block design with four replications during winter (2010-11 and 2012-13) at the Department of Botany University of Peshawar Pakistan. Overall, inoculated plant performed superior in terms of plant growth and quality over control. All plants measured parameters (Leaf chlorophyll content, seed protein, fiber and ash content, plant height, number of seed pod^{-1}, leaves plant^{-1}, flowers plant^{-1}, pods plant^{-1}, pod length and thousand seed weight) were highest in plant samples inoculated with VAM and Rhizobium in combination as compared to sole application of VAM or Rhizobium. Combined inoculation of VAM and Rhizobium caused 10, 24, 17, 21 and 14% increase in seed protein content, leaf chlorophyll content, seed fiber content, seed ash content and number of seed pod^{-1} over sole application of VAM and Rhizobium when averaged over two years. Combined application of Rhizobium + VAM enhanced seed yield plant^{-1} by 45% over control and 24% and 28% over sole inoculation of VAM and Rhizobium respectively. It is therefore concluded that dual inoculation of VAM + Rhizobium and rock phosphate may be of only limited consequence in high input agricultural systems.

Key words: Lentil, AM Fungi, *Rhizobium*, RP fertilizer, Inoculation, Chlorophyll content.

Introduction

The word Mycorrhiza is given to a mutualistic association between a fungus (Myco) and the roots (rhiza) of the plants. This association is symbiotic because the relationship is advantageous for both partners. The macrosymbiont (the plant) gains increased exploration of the soil with the intricate net of hyphae branching filaments of a fungus that increases the uptake of water and nutrients from the soil interphase. The microsymbiont (the fungus) uses the carbon provided by the plant for its physiological functions, growth and development (Davies, 2011).Amongst the mycorrhizal associations, the AM association is the most common one (Tahat and Sijam, 2012). Arbuscular mycorrhiza symbiosis is witnessed in approximately 80% of vascular plant species in all major terrestrial biomes (Feddermann *et al*., 2010; Smith *et al.,* 2010). Arbuscular mycorrhizal fungi are found in association widely under many environmental conditions (Chen *et al*., 2005; Souchie *et al.,* 2006). The advantageous influence of mycorrhizae has been reported under deficient phosphorous soil (Chen *et al.,* 2005; Duponnois, 2006). The improved nodulation and nitrogen fixation in legume crops as a result of better phosphorus nutrition was reported by Athar *et al.* (2005). Furthermore, Fonseca *et al.* (1993) also reported a better association of AM fungi with soybean for enhances nitrogenase activity. Likewise, Khan *et al.* (2008) found that the dry weight of shoot and root improved in dual inoculation while the nutrients uptake (NPK) was also higher due to dual inoculations. Bhattacharjee & Sharma (2012) also suggested that dual inoculation have the capability to increase the nutrients content and chlorophyll content of pigeon pea.

Most of the legumes possess two types of microbial symbionts namely mycorrhizal fungi and nitrogen fixing bacteria thereby establishing triple association, capable of supplying N and P contents to the plants (Silveira & Cardoso, 2004). Dual inoculation with both microorganisms results in a tripartite mutualistic symbiosis and generally increases plant growth to a greater extent than inoculation with only one (Chalk *et al*., 2006). Inoculation alone or in combination of beneficial microorganisms including AMF, rhizobia, PGPR and PSB (Phosphate Solubilizing Bacteria) have been observed to increase production in green gram and chickpea, nitrogen fixation and nutrient uptake (Jain *et al.,* 2007; Rahman *et al.,* 2008; Jain *et al.,* 2008; Ray & Valsalakumar, 2009; Pir *et al.,* 2009; Akhtar & Siddiqui, 2009; Jain *et al.,* 2009; Singh & Singh, 2010; Thenua *et al.,* 2010). Murat *et al.* (2011) reported that AMF inoculation, alone or in combination with rhizobial inoculation, increased in yield, root colonization and phosphorus content of the seed and shoot.

The research is planned to evaluate the beneficial effect of bio-fertilizer (VAM, *Rhizobium* and Rock phosphate) inoculation alone and in combination on growth and productivity of Lentil (*Lens culinaris*) and to investigate the effect of inoculation of AM fungus, *Rhizobium*and rock phosphate on chlorophyll content of lentil.

Materials and Methods

Soil analysis before the start of experiment: Three core samples were randomly taken on the entire field at 0-15cm depths before sowing. The soil samples were bulked, air-dried soon after collection and were passed through 2mm sieved mesh to remove crop residues, stones or earth worms or any unwanted material. The mechanical analysis of the soil was done by the hydrometer method (Bouycous, 1951). The percentage of sand, silt and clay was read on a textural triangle to determine the soil texture and percentage of silt, sand and clay in the experimental site. Table 1 presents the physio-chemical properties of experimental site before launching the field experiments.

Table 1. Basic soil characteristics (physico-chemical properties) before the start of experiment.

Soil physical properties	Unit	Value at the depth of (0-15cm)
Sand	%	24.39
Silt	%	67.20
Clay	%	8.44
Soil texture class	-	Silt loam
Soil bulk density	-	0.27
Soil chemical properties		
pH	-	7.6
EC	d Sm-1	0.56
Soil P	mg kg-1	2.18
Sol total N	%	0.045
Sol K	mg kg-1	83.25
Organic matter	%	0.73

Experimental design and treatments: The study was conducted during summer for two years at the department of Botany University of Peshawar Pakistan for the year 2010-2011 and 2012-2013. Seeds of *Lens culinaris* (NARC2008-4) were sown in circular earthen pots (21 cm height and 70.6cm diameter) filled with a mixture of soil which was sterilized in Laboratory of Soil and Environmental Science Department, Agricultural University Peshawar. The sample was mixed with sand in ratio of 2:1 (v/v). Rhizobia and AMF propagules were mixed. Rhizospheric soil from wheat field having high spore number of different AMF i.e., *Glomusfasciculatum, G. mosseae* and *G. aggregatum* and roots of wheat and maize infected with Arbuscular mycorrhiza were used as rhizobase inoculum. The root pieces along with soil base inoculum (rhizospheric soil) were spread uniformly in layers at a depth of 3cm and 6cm before sowing. Inoculum for each pot consisted of 180 g of mycorrhizal infected roots and adhering soil. *Rhizobium* seed inoculation was done by using effective *Rhizobium leguminosarum,* obtained from Dept. of Soil Science, National Agriculture Research Center, Islamabad. The seeds were coated with gum acacia and 10kg pot^{-1} soil was used. Soil used during the experiment was collected from the University of Peshawar. The soil was air-dried, sieved through 4 mm sieve and characteristics determined at the soil laboratory of the department of Soil and Environmental Science, The University of Agriculture, Peshawar. Characteristics of the soil were as follows; soil texture as sandy loam, organic matter 6.65 percent, nitrogen 0.044 percent and phosphorus 1.13 percent (Olsen & Sommers, 1982). The pH of saturated paste (7.30) was determined by pH meter as recommended by Richard *et al.* (1954). Seeds were inoculated individually with *Rhizobium* and AM fungi (applied as layering on soil surface) and combination of both and different levels of rock phosphate fertilizer. The plants were irrigated with tap water as and when required. The plants were collected from each replication for sampling at vegetative, flowering and fruiting stages after sowing. The data related to the plant height, pods plant^{-1} and seeds pod^{-1} was recorded on 45th day (Table 2).

Table 2. Details of the treatments used in experiment.

S. No.	Treatment	Detail
1.	Control	Nill application of inoculums
2.	VAM	VAM fungus alone
3.	RH_1	Rhizobium
4.	VAM + RH_I	VAM and Rhizobium in combination
5.	RP_1	Rock phosphate alone
6.	RP_2	Rock Phosphate + VAM
7.	RP_3	Rock phosphate + VAM + Rhizobium

Determination of chlorophyll contents and proximate analysis: Leaf chlorophyll content was measured weekly using a SPAD chlorophyll meter (SPAD-502Plus) with 15 days interval after 30 days of sowing and was averaged over two years. Kjeldhal-N was determined and protein content was calculated by multiplying nitrogen by the factor 6.25 (Anon., 2000). Percent moisture, crude fat, ash and crude fiber contents were determined using the methods of Association of Official Analytical Chemists (Anon., 2006).

Statistical analysis: Experimental data were statistically analyzed using the Statistical Analysis System program; the means were subjected to LSD test at 5% level of probability after revealing differences among treatments (Jan *et al.,* 2009).

Results

Response of lentil quality to different inoculation sources (averaged over two year): Figure 1 represents data regarding changes over time in lentil leaf chlorophyll content (two years averaged) in response to different inoculums (VAM, Rhizibium and rock phosphate alone and in combination). Overall, significant effect of treatments was observed on chlorophyll content on different intervals. Sole inoculation with VAM resulted in higher chlorophyll content as compared to Rhizobium at all intervals. The performance of VAM + Rhizobium was superior after 15 days of interval while the RP3 had the highest chlorophyll content after 30 and 45 days of intervals. Moreover, significant changes in seed protein and carbohydrate content were observed as a result of different inoculums (Fig. 1a and Fig. 1b, respectively). The seed protein content was higher in second year of the study as compared to first year regardless of the treatments application (Fig. 1a). All treatments increased seed protein content in comparison with control however; unexpectedly seed carbohydrates were reduced in all inoculated plots over control (Fig. 2b). Among treatments, combination of VAM and Rhizobium substantially increased seed protein content as compared other treatments during both the years. In contrast, seed carbohydrates were higher in untreated plots compared to inoculated plots (Fig. 2b). The effect of inoculums on seed fiber and ash content are indicated in Figures 3 (a & b). Overall, all treatments considerably influenced seed fiber content. The seed fiber content was higher for combine inoculation with VAM and Rhizobium followed by sole inoculation with Rhizobium while lesser fiber content was recorded in control plots. There were no considerable variations in seed ash content due to either sole inoculation or combined however the combination of VAM and Rhizobium resulted in higher seed ash content followed by RP_3 in both years. The ash content was higher in second year of the experiment than first year.

Response of lentil yield and yield components to inoculation sources: Data regarding yield related traits of lentil are reported in Table 3. Statistical analysis of the data indicated that considerable differences were recorded among different inoculated treatments for lentil yield contributing traits (Table 4). Plants inoculated with both VAM and RH_I had recorded substantially higher number of leaves and flowers plant-1, plant height, number and length of pods and number of seeds pod-1, however it was at par with sole inoculation either with VAM, Rizobia or RP_3 for all studied traits except number of flowers, pods and pod length for RH_I and Sole RP. The sole application of rock phosphate had resulted in lesser number of leaves, plant height, flowers, number and length of pods and number of seeds. The non-inoculated plants were short stature and had least number of pods lant^{-1}. Moreover, combined inoculation of VAM + Rhizobium enhanced seed yield plant^{-1} by 47% over control while 24 and 27% over sole inoculation of VAM and Rhizobium respectively.

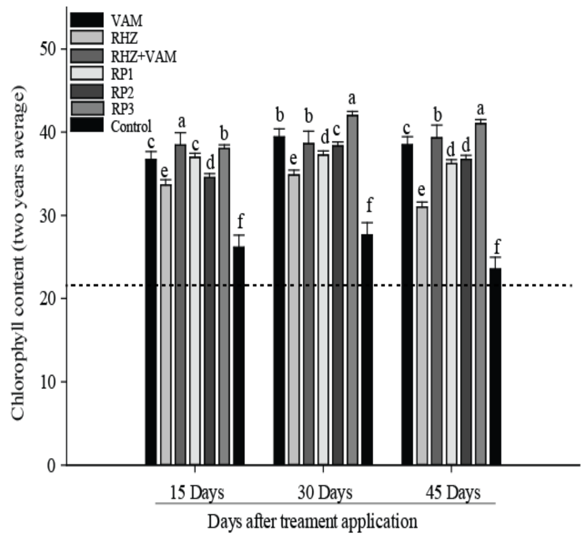

Fig. 1. Changes in lentil chlorophyll content (two years average) over time in response to the application different inoculums (Control, VAM = VAM fungus alone, RHI = Rhizobium alone, RHI + VAM = Rhizobium and VAM combined, RP1 = Rock phosphate alone, RP2 = Rock phosphate and VAM combined and RP6 = Rock phosphate + VAM + Rhizobium). Values represent means ± SEM (*n* = 6). Different letters represent significant differences among treatments (Pval<0.05). Dotted line represents the value of control pots (treated with nill inoculation).

Table 3. Plant height, number of leaves, number of flowers, number of pods plant^{-1}, pod length and number of seed pod^{-1} of lentil as affected by bio-fertilizer treatments.

Treatments	Plant height	Leaves plant^{-1}	Flowers plant^{-1}	Pods plant^{-1}	Seeds pod^{-1}	Seed yield plant^{-1} (g)
Control	29.08 bc	18.8 ab	6.00 abc	6.20 abc	1.60 ab	12 e
VAM	32.12 a	19.4 ab	6.80 ab	6.60 ab	2.00 a	24 c
RHI	30.96 a	19.4 ab	5.40 bc	5.80 bc	1.80 a	20 d
VAM + RHI	32.26 a	19.6 a	7.20 a	7.40 a	2.00 a	42 a
RP1	27.90 c	18.4 b	5.40 c	5.00 c	1.00 b	38 b
RP2	30.44 ab	19.2 ab	5.60 bc	5.40 bc	1.40 ab	36 b
RP3	31.24 a	19.6 a	6.20 abc	6.00 bc	1.60 ab	40 a
$LSD_{0.05}$	1.84	1.03	1.3162	1.32	0.67	2.35

Table 4. Mean square values for plant height, number of leaves, plant height, number of flowers, pods plant^{-1}, pod length and seed pod^{-1} of lentil as affected by bio-fertilizer treatments.

SOV	DF	Flower plant^{-1}	Plant height	Leaves plant^{-1}	Pods plant^{-1}	Pod length	Seeds pod^{-1}
Rep	4	3.400	1.632	0.686	0.686	0.032	0.543
Treat	6	2.457**	12.686**	1.000*	3.114**	0.628*	0.629*
Error	24	1.016	1.992	0.619	1.019	0.018	0.259
CV		16.57	4.62	4.10	16.67	7.93	31.28

** = Significant at 1% probability
* = Significant at 5% probability

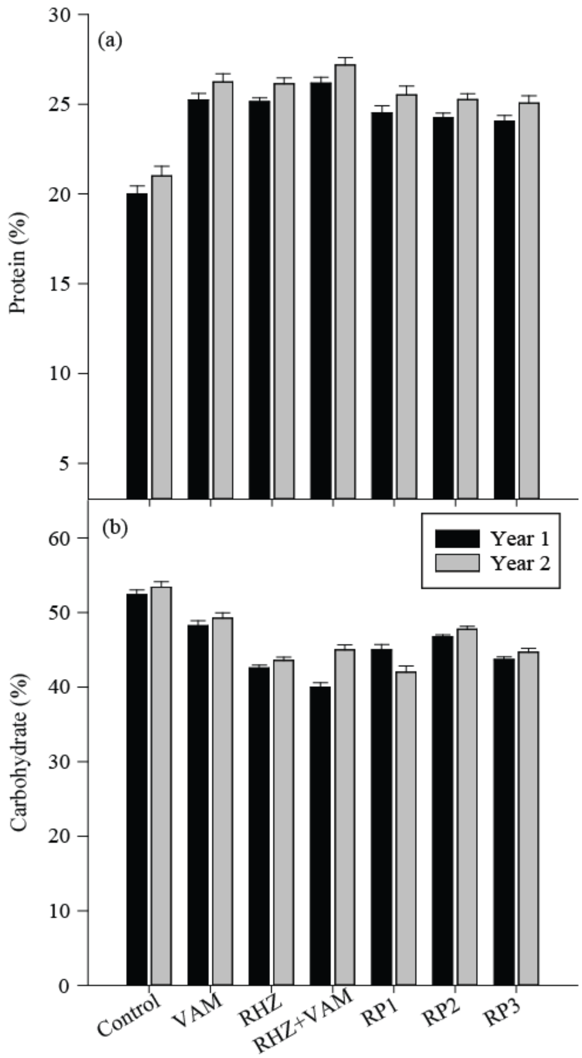

Fig. 2(a&b). Lentil Protein and carbohydrate content (%), respectively, in response to the application of different inoculums (Control, VAM = VAM fungus alone, RHI = Rhizobium alone, RHI + VAM = Rhizobium and VAM combined, RP1 = Rock phosphate alone, RP2 = Rock phosphate and VAM combined and RP6 = Rock phosphate + VAM + Rhizobium). Values represent means ± SEM (*n* = 6).

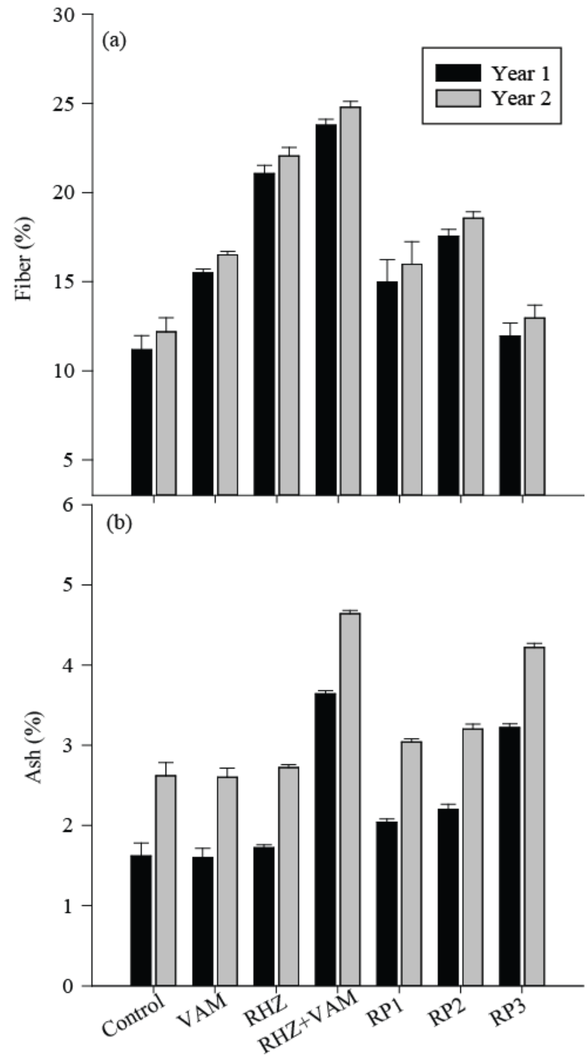

Fig. 3(a&b). Lentil seed fiber and Ash content (%), respectively, in response to the application of different inoculums (Control, VAM = VAM fungus alone, RHI = Rhizobium alone, RHI + VAM = Rhizobium and VAM combined, RP1 = Rock phosphate alone, RP2 = Rock phosphate and VAM combined and RP6 = Rock phosphate + VAM + Rhizobium). Values represent means ± SEM (*n* = 6).

Discussion

The need to augment fertilizer use efficiency and to discover the most suitable and sustainable alternative to fertilizer, to minimize crop dependency of fertilizer, is clearly evident. The use of inorganic fertilizer not only bring surge in the production cost but may also prove to be instrumental in creating a large number of environmental problems alongside. It is likely that climate change and ecosystem degradation inflict new restrictions, accordingly sustainable agriculture and organic sources of nutrients has an essential function to perform in conserving natural resources. To eliminate such like problems, the use of legume for increasing soil fertility is considered to be the only viable solution for not only increasing crop production and yield but also play an important role in ameliorating soil fertility and productivity. Furthermore, to enhance the nutrients (nitrogen and phosphorus) fixing ability of various bacteria and fungi, its essential to treated seeds with proper inoculums before sowing. The dual inoculation of seed with Vesicular Arbuscular Mycorrhiza and Rhozobia enhanced the growth and quality traits i.e. protein and oil contents (Yadav & Ashok, 2015). Similarly Researches in the past few decades on various aspects of root symbionts have shown that dual interaction of AM fungi and Rhizobium has improved the growth, nodulation and yield (Talaat and Abdallah, 2008). Similarly, seed inoculation and soil treatment also showed significantly higher protein, carbohydrates fats, crud fibers, and dry matter of soyabean than uni-nocultaed plants (Egberongbe *et al.,* 2010). Sarah & Burni (2013) also reported that arbuscular mycorrhizal fungi had showed promising effects on crude protein, fat, moisture and ash contents in mycorrhizal plants except carbohydrate. Our result are also correlate with Samanhudi *et al.* (2014) who studied the effect of AMF inoculation on Temulawak plant observed that mycorrhizal inoculation improve yield of studied plant. VAM fungi have been shown to improve the chlorophyll contents on the leaves of many plants (Abdel *et al.,* 2000; Diaz *et al.,* 2006; Zuccarni *et al.,*2007; Sampathkumar *et al.,* 2003).It is evident from our data that VAM inoculation improved plant growth expressed as plant height compared with the un-inoculated plants. Our results correlate with the findings of Lokshman & Kadam (2011) that plants inoculated with both rhizobial and mycorrhizal symbiosis improved growth, nodulation and nitrogen fixation. Our results were in-line with the findings of Jarande *et al.* (2006) who stated that treatments had higher values of growth parameters including plant height, number of seeds per plant and pod length. Our results are also in-line with the findings of (Rajasekaran & Nagarajan, 2005; Mortimer *et al*., 2007; Khan *et al*., 2008; Nishita & Joshi, 2010; Nazir *et al.,* 2011). Our findings were supported Hani (2009) who reported significant effects of bio-fertilizer on plant height. Same observations were also reported by Mortimer *et al.* (2008) stating that synergistic effects of the combined application of rhizobia and AM fungi enhance plant growth to a greater extent than singular inoculation. Our result agree with the finding of Jia *et al.* (2004) who reported that inoculation with AM fungi promoted biomass production and photosynthetic rates in *Vicia faba* because of the enhanced P supply due to AM fungi inoculation. Our findings were in-line with Hussain *et al.* (2010) who studied the influence of phosphorus fertilization and *Rhizobium* inoculation on growth and yield parameters of mungbean. The present results indicated that VAM produced significantly better growth attributes when combined with RP or Rhz. as compared to the yield and yield attributes over rest of the bio-fertilizers and fertilizer (RP) alone. The significant increase in number of leaves per plant, plant height, pods plant and seeds per plant due to the either single or dual inoculation of seed with VAM and RBI was also supported by the findings of Balachandran *et al.* (2005) who reported that the effect of inoculation with *Rhizobium* and phosphate solubilizing bacteria (PSB) significantly increased plant height, number of leaves and leaf area. The highest numbers of seeds pod^{-1} were found in either dual or single inoculation than control. These findings coincide with those of Jagvir *et al.* (2004) and Lokshman & Kadam (2011) who concluded that dual inoculation, *Rhizobium* and VAM resulted in increase in grain yield, over no inoculation. Similar results were obtained by Jarande *et al.* (2006) who stated that treatments which included RP and seed treatment with PSB and *Rhizobium* application of RP and PSB + *Rhizobium* recorded higher values of growth as well as yield. Our results are also supported by Meghvansi & Mahna (2009), who found that dual inoculation of *rhizobium* + VAM was superior over single inoculation. Similarly our results correlate with Killani (2010); Olawuyi *et al.* (2011) and David *et al.* (2008). It is evident from the results that the efficacy of VAM fungi was influenced by co-inoculation with Rhizobium and Rock phosphate increased the chlorophyll contents due to dual inoculation that might help in increasing the rate of photosynthesis and ultimately increase the plant growth.

Conclusion

It is concluded that lentil should preferably be grown with dual inoculation of VAM along with *Rhizobium* and rock phosphate. The present finding showed that triple inoculation also exhibited significantly positive response to growth of lentil. Mixed VAM + *Rhizobium* + rock phosphate had increased yield of lentil. The dual inoculation of AM fungus, Rhizobium and Rock phosphate also enhanced chlorophyll contents of plant indicating that their combination may have a potential role to enhance the crop productivity.

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