

EFFICIENCY OF PLANT GROWTH PROMOTING MICROORGANISMS ON THE GROWTH PERFORMANCE OF PADDY RICE

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Abstract

Plant Growth Promoting Microorganisms (PGPMs) are beneficial organisms found in the plant eco-systems and enhance plant growth. Their ability to solubilize the unavailable soil phosphorus, increase the uptake of nitrogen and synthesize growth promoting hormones such as auxins puts them in a good position to be utilized as bio-fertilizers. It has been documented that the rice plant rhizosphere is a habitat for various microorganisms which act as bio-fertilizers. It has been reported that inoculation of rice plants with these microbes resulted in increased plant growth. The present study was undertaken to assess the effectiveness of selected microorganism isolates as rice seedling promoters under field conditions. Prior to inoculation, the microbial isolates had been screened for their physiological properties as PGPMs. To investigate the effects of PGPMs inoculants on the growth of Basmati 317 rice plants, field experiments were conducted at Jomo Kenyatta University of Agriculture and Technology in the period 2013 to 2014. The experimental design was split-plot with four replications. Data was collected on growth attribute parameters including plant height, root length, tiller numbers, shoot dry weight and root dry weight. Analysis of variance procedure for split-plot design was done, and the Duncan multiple range test was used for separating the treatment means. The main-plots (concentrations) as well as the sub-plots (inoculants) treatments were significantly different at $p < 0.005$ in all the parameters tested. Interaction between concentrations and inoculants was only found in the root length. Plants inoculated with *Bacillus*, *Enterobacter*, *Staphylococcus*, *Brevudimonas*, *Pseudomonas*, *Trichoderma*, *Penicillium*, *Aspergillus* and mixed bacteria performed better than the uninoculated plants whereas best results were obtained with plants applied with chemical fertilizers. From the results, we can conclude that the use of these PGPMs as inoculant bio-fertilizers is therefore beneficial for rice production since they enhanced the growth of rice plants.

Key words: Plant growth promoting micro-organisms; Inoculation; concentrations; inoculants

1.0 Introduction

Rice (*Oryza sativa* L.) is amongst the top three most important cereal crops in Kenya. In many developing countries, rice is the most popular staple food and it feeds more than 50% of the world's population (Zeigler and Barclay, 2008). The demand for rice in Kenya is increasing at a faster rate as a result of increase in human population as well as changes in eating habit. There is therefore a need to increase rice production so as to meet the increasing demand. The most important prerequisite for improved rice production is by the use of chemical fertilizers. These fertilizers may cause environmental pollution, depletion of soil quality and reduction / elimination of soil microbes involved in paddy growth among other problems (Saharan and Nehra, 2011). Hence the need to consider using other forms of fertilizers which are eco-friendly and which can improve rice growth and production.

Sustainable rice production can be achieved through improvement in agricultural productivity such as the use of indigenous microorganisms and less dependency on fertilizers. Microorganisms which are beneficial are known to be important in paddy production. Beneficial microorganisms such as plant growth promoting bacteria (PGPB) and plant growth promoting fungi (PGPF) play a major role in maintaining ecosystem functions (Hermosa *et al.*, 2011). Plant growth promoting bacteria benefit plants by different mechanisms such as the conversion of insoluble P to accessible form which is an important trait in improving growth and increasing plant yields (Rodriguez *et al.*, 2006). The rhizospheric bacteria referred to as plant growth promoting rhizobacteria (PGPR) have the ability to solubilize phosphorus (P), fix nitrogen (N), produce phytohormones and siderophores, which can improve plant growth (Vessey, 2003). A group of beneficial fungi (PGPF) produce hormones and degrade / mineralize substrates which have the ability to enhance plant growth (Nenwani *et al.*, 2010). These beneficial microorganisms improve soil fertility through fixation of N, solubilization of P and production of phytohormones and thereafter availing these nutrients to plants (Brandl *et al.*, 1996).

Sustainable agriculture may be attained through N fixation and plant growth enhancement by microorganisms. Large amounts of mineral nutrients such as N is required by cereals for their growth, development and grain

production (Baset Mia and Shamsuddin, 2010). Among the beneficial microorganisms, bacteria and archaea have the ability to reduce and derive significant amount of N from the atmospheric reservoir to enrich the soil (Saharan and Nehra, 2011). Phosphorus has a defined role in plant metabolism which includes root development, photosynthesis and nutrient transportation within the plant (Rasipour and Asgharzadeh, 2007) thus P is an essential nutrient for rice growth and development. Auxin / Indole acetic acid (IAA) plays an active role in increasing plant growth through initiation of root elongation, cell expansion, vascular differentiation and flower initiation (Brandi *et al.*, 1996). The endogenous IAA is produced naturally by plants whereas the microorganisms produce exogenous IAA which also affects the plants' vegetative and reproductive growth (Agustian *et al.*, 2010).

The application of beneficial microorganisms to act as bio-fertilizers may enhance plant growth. According to Vessey (2003), a substance containing live microbes and which can stimulate plant growth, improve soil fertility, increase crop production without causing detrimental effects to the environment when applied to plant or soil is known as bio-fertilizer. The application of bacterial fertilizer to promote growth has been reported by Thakuria *et al.* (2004) to have benefits which include nutrient solubilization, phytohormone production and nitrogen fixation. Inoculation of plants with beneficial microorganisms has been exploited by many researchers. Through the integrated nutrient supply, the plant-microbes interaction has a major role in sustainable agriculture.

The most essential nutrients required for the growth and development of both plants and beneficial microorganisms are N and P (Vikram, 2007). According to Vikram (2007), a lot of work has been done on phosphate solubilizing microorganisms (PSM) and the results showed that inoculating crops with these organisms improved plants growth. For a long time bio-fertilizers such as *Azospirillum* sp, *Azotobacter* sp, *Rhizobium* sp, *Nitrosomonas* sp, and *Nitrobacter* sp have been in use and *Azospirillum* has been reported by Bashan *et al.* (2004) to significantly increase total plant biomass, plant height, leaf size, leaf area index and root length of cereals. Also used as bio-fertilizer as an alternative to chemical fertilizer for different crops is the PGPRs (Egamberdiyeva and Hoflich, 2004) which are capable of improving plant root length.

Due to their effectiveness in promoting growth of many crops (Thakuria *et al.*, 2004) there has been a growing interest in PGPR. However, in Kenya, there is very little information on the use of beneficial microorganisms such as PGPMs as bio-fertilizers in rice production. Therefore the present study was carried out to investigate the effect of PGPMs on the growth performance of paddy rice.

2.0 Materials and Methods

To assess the effectiveness of microbial inoculants as plant growth promoters, field experiments were conducted at JKUAT during 2013 and 2014. Situated 36Km N-E of Nairobi, the site is 1530m above sea level (Batjes, 2006) and it lies between 3° 35" and 1°45"S (latitudes) and 36° 35" and 37° 25" E (longitudes).

Prior to inoculation, nine bacterial and four fungal strains obtained from rice growing regions of Kenya (Mwea, Western and Coast) were selected based on their physiological properties as N fixers, P solubilizers and IAA producers as shown in table 1.

Table 8: Properties of selected microbial strains

Bacterial isolates	P solubilization	N fixation	IAA production
1	+	+	++
2	+	+	++
3	+++	+	-
4	++	+	++
5	+++	+	+
6	++	+	-
7	+++	+++	+
8	+++	+	++
9	+++	+	+
Fungal isolates			
10	+	-	+
11	++	-	-
12	++	-	-
13	-	-	++

Key: (+) Moderate, (++) High, (+++) Very high, (-) Negative

Sterilized Basmati 317 rice seeds were used during the study with three different microbial / inocula concentrations (10^5 cfu spores⁻¹ ml⁻¹, 10^7 cfu spores⁻¹ ml⁻¹ and 10^9 cfu spores⁻¹ ml⁻¹). The rice seeds were inoculated by immersion in the appropriate PGP suspension for 30 min, air-dried and sown immediately. The treatments were nine bacterial strains, four fungal strains, three mixed strains (i.e., all nine bacterial strains (MB), all four fungal strains (MF) and a mix of all the bacterial and fungal strains (MBF), and two controls which were the natural condition (C) and farmers' practice (C2). To obtain mixtures of both bacterial and fungal cultures, an equal volume of the cultures were mixed. Control seeds were treated with distilled water. The experiment was laid in split-plot design with four replications. The microbial concentrations formed the main plots whereas the sub-plots contained the microbial strains.

Each two kg plastic pots (15cm diameter) containing vertisols soil were planted with six treated seeds. Seven (7) days after planting, thinning was done to four seeds / pot. For the farmers' practice, two doses of fertilizers were applied. The 1st application of Di- ammonium phosphate (DAP) 125kg / ha and Muriate of potash (MOP) 75kg / ha was done three weeks after planting, while the 2nd application of Sulphate of ammonium (SA) 250kg / ha was done 7 weeks after planting. During the growth period, the weeds were removed manually and the pots throughout the experimentation period were maintained under paddy conditions.

At maturity stage, the plants were uprooted and the soil washed carefully to ensure the roots were intact. The shoots were separated from the roots and characteristics such as plant height, root length and numbers of tillers were recorded. After drying in an oven for 24 hours at 70°C, the shoot and root dry weights were taken. The data was subjected to statistical analysis of variance and significant differences among the treatment means separated by DMRT at $p < 0.05$.

3.0 Results

Table 2 shows the inoculation effects of three different concentrations (10^9 cfu spores⁻¹ ml⁻¹, 10^7 cfu spores⁻¹ ml⁻¹ and 10^5 cfu spores⁻¹ ml⁻¹) and various isolates on the rice plants' growth attributes.

3.1 Plant Height

The main plot effect for this parameter showed that plants which were treated with concentration 1 (10^9 cfu spores⁻¹ ml⁻¹) were significantly different at $p < 0.05$ from plants treated with concentrations 2 and 3 (10^7 cfu spores⁻¹ ml⁻¹ and 10^5 cfu spores⁻¹ ml⁻¹) respectively (Table 2). Plants treated with concentrations 2 and 3 had no significant differences in their heights as shown in table 2. Despite of no significant differences in heights of plants for concentrations 2 and 3, plants treated with concentration 2 were taller (71.34 cm) than those treated with concentration 3 (70.08 cm). The tallest plants (75.23 cm) were those treated with concentration 1.

There were significant differences for the sub-plot treatments as revealed in table 2 where the plants' mean heights ranged from 77.63 cm to 66.87 cm for MB and B3 treatments respectively. There were no significant differences observed for all the other treatments including the uninoculated controls (C). As shown in table 2, treatments B2, B7 and F2 performed better than treatment C2 (74.08 cm) despite of no significant differences among the treatments. No significant ($p < 0.05$) interaction was observed between the concentration and inocula for plant height.

3.2 Root Length

The main-plot treatments for concentrations 1 and 2 differed significantly ($p < 0.05$) from those of treatment 3 as indicated in table 2. Despite of no significant differences, roots of plants treated with concentration 1 were longer (9.06 cm) than those treated with concentration 2 (8.96 cm). Plants with shortest roots (8.21 cm) were those treated with concentration 3.

As for the main-plot, the sub-plot treatments were also significantly different. There was a wide range of variations in the root lengths which ranged from 7.17 cm to 11.37 cm (B2 and C2, respectively) where the uninoculated plants (C) recorded a mean root length of 9.38 cm (Table 2). Despite there being no significant differences between treatments B7, F1, F4 and C, the performance of these treatments was better than that of the uninoculated controls (10.30 cm, 9.82 cm and 9.96 cm respectively, against 9.38 cm). The concentration by inocula interaction was significant ($p < 0.05$).

3.3 Number of Tillers

The treatment effect of the main-plot for the number of tillers differed significantly ($p < 0.05$). As shown in table 2, plants treated with concentration 2 had the most number of tillers (6.36) followed by those treated with concentration 1 then those plants treated with concentration 3 (5.85 and 5.54) respectively.

Significant differences in the number of tillers was observed for the sub-plots where the C2 treated plants recorded the most number of tillers (7.33) as opposed to the MBF treated plants which had the least numbers of tillers (4.67). Apart from treatment MBF, others which had fewer tillers than the C (5.42) treatment were B1 and F1 (5.17 and 5.33) respectively (Table 2). There was no significant difference ($p < 0.05$) in the interaction between the concentrations and inocula.

Table 2: Effect of inoculation on growth attributes of rice plants

	Treatment	Plant height (cm)	Root length (cm)	Number of Tillers
Concentration				
Conc 1	10^9 cfu spores ⁻¹ ml ⁻¹	75.23a	9.06a	5.85ab
Conc 2	10^7 cfu spores ⁻¹ ml ⁻¹	71.34b	8.96a	6.36b
Conc 3	10^5 cfu spores ⁻¹ ml ⁻¹	70.08b	8.21b	5.54a
	SE (+/-)	1.6	0.719	0.339
Isolate				
	Inoculant			
B1	<i>Micrococcus yunnanensis</i>	70.60ab	8.79abc	5.17ab
B2	<i>Brevudimonas naejangsanensis</i>	76.18ab	7.17a	6.33abc
B3	<i>Acinetobacter pittii</i>	66.87a	7.39ab	5.83abc
B4	<i>Staphylococcus saprophyticus</i>	73.69ab	9.24abc	6.08abc
B5	<i>Enterobacter aerogenes</i>	70.63ab	7.75ab	5.92abc
B6	<i>Pseudomonas plecoglossicida</i>	69.86ab	7.29ab	6.33abc
B7	<i>Bacillus tequilensis</i>	76.83ab	10.30bc	6.33abc
B8	<i>Bacillus stratophericus</i>	70.53ab	7.71ab	5.58abc
B9	<i>Enterobacter cancerogenus</i>	71.88ab	8.7abc	6.17abc
F1	<i>Penicillium chrysogenum</i>	69.35ab	9.82abc	5.33ab
F2	<i>Trichoderma harzianum</i>	74.19ab	7.51ab	5.50abc
F3	<i>Penicillium pinophilum</i>	73.57ab	9.29abc	6.58bc
F4	<i>Aspergillus oryzae</i>	73.63ab	9.96abc	5.67abc
MB	MB	77.63b	8.95abc	6.50abc
MF	MF	70.66ab	8.87abc	5.75abc
MBF	MBF	68.78ab	7.90ab	4.67a
C	Cont 1	70.99ab	9.38abc	5.42ab
C2	Cont 2	74.08ab	11.37c	7.33c
	SE (+/-)	4.222	1.261	0.776
Interaction		NS	S	NS

NS = Not significant at 5% level; S = Significant at 5% level

The effect of inoculating three different concentrations (10^9 cfu spores⁻¹ ml⁻¹, 10^7 cfu spores⁻¹ ml⁻¹ and 10^5 cfu spores⁻¹ ml⁻¹) on dry matters accumulation of the rice plants is shown in table 3.

3.4 Shoot Dry Weight

In response to the main-plot treatments, a significant ($p < 0.05$) increase in shoot dry matter was observed (Table 3). Plants treated with concentrations 1 and 2 (9.38 g and 9.41 g) differed significantly in their shoot dry weights as compared to those treated with concentration 3 (8.61 g).

In the sub-plots, significant differences were also evident as shown in table 3. The C2 treated plants recorded the highest shoot dry weight (13.54 g) followed by MB and B7 treatments (11.70 g and 10.92 g) whereas the

lowest shoot dry weights were those of treatments F1 and B1 (7.13 g and 7.14 g) respectively. As shown in table 3, there was no significant interaction between the concentrations and the inocula.

3.5 Root Dry Weight

There were significant differences ($p < 0.05$) in the main-plots for the root dry weights. Concentrations 2 and 3 treated plants (1.92 g and 1.62 g) performed better than plants inoculated with concentration 1 (1.39 g) as shown in table 3.

As in the main-plots, the sub-plots showed significant differences in their root dry weights in response to different inoculants. As opposed to the rest of the treatments, the C2 treatment had the highest mean dry weight of 2.81 g (Table 3). There were no significant differences between all the other treatments and the uninoculated plants (controls). Despite of no significant differences, the B3 treated plants had the least dry weight of 1.28 g while the F4 treated plants had the highest weight (2.01 g) whereas the uninoculated plants recorded 1.61 g. No significant interaction was observed between the concentrations and inocula.

Table 3: Effect of inoculation on dry matter accumulation of rice plants

	Treatment	Shoot dry weight (g)	Root dry weight (g)
Concentration			
Conc 1	10^9 cfu spores ⁻¹ ml ⁻¹	9.38a	1.39a
Conc 2	10^7 cfu spores ⁻¹ ml ⁻¹	9.41a	1.92b
Conc 3	10^5 cfu spores ⁻¹ ml ⁻¹	8.61b	1.62c
	SE (+/-)	0.664	0.145
Isolate			
	Inoculant		
B1	<i>Micrococcus yunnanensis</i>	7.14a	1.30a
B2	<i>Brevudimonas naejangsanensis</i>	9.36abc	1.43a
B3	<i>Acinetobacter pittii</i>	7.97ab	1.28a
B4	<i>Staphylococcus saprophyticus</i>	10.21abc	1.81a
B5	<i>Enterobacter aerogenes</i>	8.48abc	1.44a
B6	<i>Pseudomonas plecoglossicida</i>	8.75abc	1.64a
B7	<i>Bacillus tequilensis</i>	10.92bcd	1.62a
B8	<i>Bacillus stratophericus</i>	8.43abc	1.58a
B9	<i>Enterobacter cancerogenus</i>	9.35abc	1.68a
F1	<i>Penicillium chrysogenum</i>	7.13a	1.36a
F2	<i>Trichoderma harzianum</i>	8.32abc	1.38a
F3	<i>Penicillium pinophilum</i>	9.45abc	1.66a
F4	<i>Aspergillus oryzae</i>	10.03abc	2.01a
MB	MB	11.70cd	1.93a
MF	MF	8.38abc	1.43a
MBF	MBF	7.08a	1.64a
C	Cont 1	8.21abc	1.61a
C2	Cont 2	13.54d	2.81b
	SE (+/-)	1.522	0.312
Interaction		NS	NS

NS = Not significant at 5% level

4.0 Discussion

The present study which involved the inoculation of rice with selected bacterial and fungal isolates based on their physiological properties as growth enhancers led to different results of the tested parameters in

comparison with the controls. The effect of the isolates as inoculants on rice growth in this study was assessed in three different concentrations. The main-plot results revealed that the performance of concentrations 1 and 2 (10^9 cfu spores⁻¹ ml⁻¹ and 10^7 cfu spores⁻¹ ml⁻¹) was better than that of concentration 3 (10^5 cfu spores⁻¹ ml⁻¹) for all the tested parameters apart from root dry weight (Tables 2 and 3). In their studies, Zehnder *et al.* (2001) also reported that effective root colonization levels are achieved through the application of high numbers of microorganisms. In the rhizosphere, the establishment of an active cell population density of PGPMs is a prerequisite for their efficacy. Thus PGPMs suspensions are prepared at densities of 10^8 to 10^9 cfu ml⁻¹. The inconsistency in the field results regarding the application of PGPMs is likely to be caused by the rapid decline of the microorganisms' population as they undergo competition among different microorganisms present in the non-sterile soil (Martínez-Viveros *et al.*, 2010).

The PGPMs significantly influenced all the growth parameters of the rice plants. Similar results were reported by Ahmed *et al.* (2011) who observed increases in rice plant growth parameters after application of bio-fertilizers. Minorsky (2008) showed colonization of roots of various plants by *Pseudomonas fluorescens* isolated from the roots of graminaceous plants to increase height, flower number and fruit number amongst other parameters. Mia *et al.* (2009) reported increased plant growth as a result of inoculating cereals with PGPMs which they suggested acted as bio-fertilizers.

Vegetative growth phase determines the quantity of biomass produced by plants hence it is the most vital growth phase of any crop. The development and increase in the number of tillers is regulated by this phase.

According to Sharma *et al.* (2014) products of a vigorous vegetative crop growth include higher plant height, larger number of leaves and tillers and higher root and shoot dry mass. As evidenced in this study, in response to microbial inoculation, plant height, root length, tiller number and root and shoot dry weights of the some rice plants were higher in comparison to the uninoculated plants (Tables 2 and 3). In this study for the tested growth attributes, nine isolates namely; *Bacillus tequilensis*, *Enterobacter cancerogenus*, *Staphylococcus saprophyticus*, *Brevudimonas naejangsanensis*, *Pseudomonas plecoglossicida*, *Trichoderma harzianum*, *Penicillium pinophilum*, *Aspergillus oryzae* and Mixed Bacteria performed better than the uninoculated controls(C). As reported by Kaymak *et al.* (2008) species of *Bacillus* present in the immediate environs of plant roots maintain contact with plants thus enhancing their growth through the improvement of various root parameters hence can be used as bio-fertilizers. The growth of various plants has been stimulated by *Pseudomonades* and specific strains have been used as seed inoculants on plants (Johri, 2001). Studies have revealed that the genus *Enterobacter* which is allied to the rhizosphere plays an essential role as nitrogen fixers thus exerting a valuable effect on plant growth (Tilak *et al.*, 2005). To improve growth of rice Ashrafuzzaman *et al.* (2009) isolated 10 PGPR strains most of which were significant in increasing plant height, root length and dry matter of roots and shoots of plants. Mishra and Sinha (2000) examined four fungal strains (*Gliocladium virens*, *Trichoderma virens*, *T. harzianum* and *Aspergillus niger*) for their effect on rice growth parameters. They reported significant increase in root length, shoot height and fresh weight of the seedlings.

In comparison with the uninoculated controls, the increase in the root and shoot growth as a result of microbial inoculation shows the beneficial role of these PGPMs. It has earlier been reported by Gunes *et al.* (2009) and Karlidag *et al.* (2011) that seed inoculation by rhizobacteria has improving effect on shoot dry weight. This growth enhancement is accredited to the ability of these microorganisms to fix nitrogen, solubilize phosphate and to produce the growth promoting hormones which include IAA (Salantur *et al.*, 2006).

In this study it was also evident that plants treated with Mixed Bacteria (MB) had greater values in plant height, number of tillers and shoot and root dry weights compared to the uninoculated control plants (C). Similar to these results are the findings of Naseriad *et al.* (2011) who demonstrated that the highest plant height and stem diameter in comparison with other treatments was in response to the double inoculation of *Azotobacter* and *Azospirillum*. Mixed inoculants interact synergistically thus providing nutrients and stimulating each other either through physical or biochemical activities hence boosting beneficial traits including nitrogen fixation. As reported by Bashan (1998), mixed inoculation of plants with bacteria and fungi produces synergistic interactions that effect significant improvement in plants growth and in the mineral nutrient uptake of P and N amongst others.

The mechanisms employed by microorganisms to stimulate plant growth is not known, however phytohormones production, phosphate solubilization and mineral nutrient uptake promotion are thought to be involved according to Ashraffuzzaman *et al.* (2009). Among the mechanisms involved in plant growth promotion is the production of plant growth regulators which are signal molecules and act as chemical

messengers. According to Martinez-Viveros *et al.* (2010) these growth regulators play a vital role in plants growth and development. Also reported by Spaepen *et al.* (2007) is that out of the six pathways for auxin biosynthesis, five of them depend on tryptophan as the core precursor. Through acidification, chelation, exchange reactions and gluconic acid production processes (Gulati *et al.*, 2010) P solubilizing bacteria can convert insoluble phosphates to forms accessible to plants.

The PGPMs promote plant growth through N fixation, availing P for uptake by plant and production of phytohormones (Tortora *et al.*, 2011). Bodker *et al.* (1998) reported that *Pseudomonas* have various PGPR traits such as enhanced IAA production while fungi improve P uptake. Cakmakc *et al.* (2006) showed that *Pseudomonas* and *Azospirillum* had the potential for agricultural exploitation and therefore can be utilized as natural fertilizers.

5.0 Conclusion

As revealed by the present study, the P solubilizing, N fixing and IAA producing PGPMs stimulated the vegetative growth as well as increasing the dry matter accumulation of the rice plants. Therefore these results imply that the improvement in the vegetative growth and dry matter accumulation of the rice plants by application of PGPMs as inoculant bio-fertilizers is probably due phosphorus solubilization, nitrogen fixation and induction of IAA production. Therefore the present study suggest that the use of PGPMs such as *Bacillus* sp, *Enterobacter* sp, *Staphylococcus* sp, *Brevudimonas* sp, *Pseudomonas* sp, *Trichoderma* sp, *Penicillium* sp, *Aspergillus* sp and Mixed bacteria as inoculant bio-fertilizers can be a promising substitute to inorganic fertilizers for sustainable rice production in Kenya.

Acknowledgements

The authors are thankful to the National Commission for Science, Technology and Innovation (NACOSTI) for financial assistance. We also appreciate the departments of Horticulture and Botany (JKUAT) for availing laboratory facilities for the study.

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