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Exogenous Putrescine Enhances Rice Seed Germination in Salinity Stress

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ABSTRACT— *Putrescine plays a distinct role in various types of biological functions in all living organisms, as well as environmental stress. The aim of the study is to effect of exogenous putrescine on the physiological condition of the rice seeds investigated. Salt treatment resulted in the reduction of speed of germination, germination, germination energy, germination energy percentage, final germination percentage, shoot and root length, and plant dry weight, where proline content was remarkably increased. Among the rice varieties, BRRI 48 was affected maximum by way of salinity in case of germination and growth, and GHORIAL become least affected in comparison to other varieties. However, exogenous putrescine treatment significantly enhances rice seeds germination, as well as shoot and root length in all varieties. Our findings reveal that plant putrescine tremendously reduced rice salt tolerance.*

Keywords— Rice, Polyamine, Putrescine, Salt Stress, Proline.

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1. INTRODUCTION

Rice (*Oryza sativa*) is the main cereal harvests of the world and the greater part of total populace utilized as a staple food. Rice gives around 76% of the complete calories and 66% of the protein admission of individuals' eating routine. There is in excess of 110 nations on the planet involving rice very nearly 160 million hectares, 700 million tons rice delivering consistently. Rice is the overwhelming harvest in the Asia-Pacific district. The area creates and consumes north of 90% of the world's rice [1].

Salinity is one of the abiotic pressure elements that motive vital troubles in crop production. Salinity begins to reveal its effect from seed germination, and whilst the plant is over this level, it may purpose full harm to the plant if the salinity impact will increase. For this reason, it's far essential to recognize the responses of crops to salinity in phrases of continuity of production. Improving the tolerance of flora to unfavorable situations is a main trouble for agricultural productivity and additionally essential for environmental sustainability due to the fact vegetation with low stress tolerance use extra water and fertilizer [2]. Saline soils are mainly huge- unfold alongside the coast of the dry and semi-dry areas inside the global in which the quantity of rainfall is inadequate to leach the mineral salts out of the basis-sector [3]. Due to salt strain, antioxidant enzymes, chloroplast and cytosol enzyme activities had been determined to be essential changes in plant life [4].

Polyamines (PAs) are aliphatic nitrogenous bases having low molecular weights containing or extra amino corporations, and that they have effective biological pastime [5]. They are widely distributed in eukaryotic and prokaryotic cells [6, 7]. In large plants, PAs are especially found in their natural condition. spermidine (Spd), putrescine (Put), and spermine (Spm) are the principle PAs in vegetation, and they may be involved in the law of numerous physiological techniques [8], including flower improvement, embryogenesis, organogenesis [9], senescence, and fruit maturation and improvement. In well-known, PAs also are seemed as regulators in the process of embryogenesis in each angiosperms and gymnosperms [10]. PAs are typically amassed in abiotic strain. Shao [11] discovered that warmness tolerant alfalfa cultivar contained higher stage of Spd.

Although PAs have been cautioned in enhancing salt strain tolerance in rice, the jobs of endogenous PAs continue to be unknown. In this take a look at, we explored the impact of salt stress on rice and the biochemical adjustments have been additionally investigated. To mitigate the terrible outcomes of salt stress on rice putrescine became carried out to the rice seeds.

2. MATERIALS AND METHODS

2.1 Seeds sterilization and Salt treatment

Seeds of four rice varieties (BRRI 48, BRRI 36, BRRI 28 and GHORIAL) were surface sterilized with 70% ethanol for 5 min, sodium hypochlorite (NaOCl) for 10 min and 0.1% (v/v) mercuric chloride ($HgCl_2$) for 5 min [12]. After expansive washing, Seeds were then germinated in petri-dishes treated with 0 (control/mock), 100, and 150 mM/L NaCl (saline conditions) at 37°C for 5 days.

2.2 Essay of germination parameters

After conclusive count, speed of germination (SG), final germination percentage (FGP), germination energy percent (GE%) were determined by following the accompanying formulae [13].

$SG = N_2/D_2 + N_3/D_3 + \ldots \ldots + N_i/D_i$

$FGP = S/T \times 100$

$GE\% = N/T \times 100$

Here, S is the quantity of last sprouted seeds, T is the all-out number of seeds, N is the quantity of developed seeds and N_i is the quantity of sprouted seeds each day (D_i).

2.3 Measurement of dry weight

The sterilized seeds were aseptically grown in petri-dishes treated with 0, 100, and 150 mM NaCl solution with solid 25% MS medium were transferred to a plant growth chamber programmed the cycles of 16 h light/8 h dark for 7 days as described by [14]. The relative humidity was 60/80%, and the temperature was maintained at 28°C during the day and 26°C during the night. After 7 days, plants were washing with deionized water and randomly selected seedlings for dry weight was recorded after oven drying at 80°C to constant weight.

2.4 Determination of proline content

Complementary proline content was measured by following the method of [15]. Fresh root and shoot samples were homogenized in 3% sulpho-salicylic acid by using a mortar and pestle. Then the extract was taken in test tube and the addition of glacial acetic acid followed by adding ninhydrin reagent. After that the reaction mixture was boiled in a water bath at 100° C for 30 minutes. After cooling, required volume of toluene was added. After thorough mixing, the absorbance of a chromophore containing toluene was measured at 520 nm against toluene black on UV visible spectrophotometer. The proline content was measured by using calibration curve and expressed as mg proline per g fresh weight of tissue.

2.5 Putrescine treatment

Experiments were performed with four rice varieties were grown in petri-dishes treated with150 mM NaCl solution with solid 25% MS medium and along with or without 5 mM putrescine in a plant development chamber that was the cycles of 16 h light/8 h dark for 5 days. After 5 days, seeds germination, root and shoot weight were measured

3. RESULT AND DISCUSSION

3.1 Impact of salt stress on rice seed germination

Seed germination percentages were oppositely related to salt concentration level. Speed of germination were decreased in all varieties when the salinity levels increased (Table 1). In mock, the germination percentages of all varieties were decreased under 100 mM and 150 mM salt treatment at 5 days. Speed of germination of BRRI 48 was decreased by 55 and 73% under 100 mM and 150 mM salt treatment at 5 days respectively (Table 1). While BRRI 37 was decreased by 47 and 70%, BRRI 28 was decreased by 48 and 70%, and GHORIAL was decreased by 52 and 64% under 100 mM and 150 mM salt treatment at 5 days respectively (Table 1). Table 2 showed that germination, germination energy, germination energy percentage, and final germination percentage of rice varieties BRRI 48, BRRI 37, BRRI 28, and GHORIAL were decreased under 100 mM and 150 mM salt treatment at 2, 3, 4, and, 5 days respectively. Final germination percentage of BRRI 48 were decreased by 54 and 68%, BRRI 37 was decreased by 46 and 64%, BRRI 28 was decreased by 42 and 64%, and GHORIAL was decreased by 45 and 62% under 100 mM and 150 mM salt treatment at 5 days respectively (Table 2). Salt stress inhibits plant growth due to osmotic and ionic properties on soil solution [16]. Immense levels of soil saline condition can substantially reduce different varieties of seed sprouting and seedling growth, due to the connected effects of tremendous osmotic capability and distinct ion toxicity [17]. Immense aggregation of Na+ in salt stress causes menacing of water potential and produces the plant incapable to extract water from soil osmotic stress [18]. These data concluded that salt stress constrained rice seed germination.

Table 1 Impact of salinity on speed of germination of rice varieties

Salt treatment	Day (D)	Germination (N)				N/D				Speed of germination, SG=(N2/D2+N3/D3+N4/D4+N5/D5)			
		BRRI 48	BRRI 36	BRRI 28	GHORIAL	BRRI 48	BRRI 36	BRRI 28	GHORIAL	BRRI 48	BRRI 36	BRRI 28	GHORIAL
Mock	2	10.67	9.33	6	8	5.33	4.66	3	4	48.29	37.89	33.94	31.03
	3	38.67	28.67	26	22	12.89	9.56	8.67	7.34				
	4	60	44	42.67	36.67	15	11	10.67	9.16				
	5	75.34	63.34	58	52.67	15.06	12.67	11.6	10.53				
100 mM salt	2	2.67	2	0.67	2	1.34	1	0.34	1	21.77	20.08	17.78	14.96
	3	20	17.34	15.34	10.67	6.67	5.78	5.11	3.56				
	4	27.33	26	22.67	18.67	6.84	6.5	5.67	4.67				
	5	34.67	34	33.34	28.67	6.93	6.8	6.67	5.73				
150 mM salt	2	1.34	0.67	0	0.67	0.67	0.34	0	0.34	12.97	11.43	10.07	9.72
	3	10	8.67	7.34	6.67	3.34	2.89	2.45	2.23				
	4	16.67	14.67	14	12.67	4.17	3.67	3.5	3.17				
	5	24	22.67	20.67	20	4.8	4.53	4.14	4				

Note: Rice seeds were treated with 100 or 150 mM NaCl for 2, 3, 4, and 5 d. Results are mean values of three replicates.

Table 2 Impact of salinity on germination energy, germination energy percentage, and final germination percentage of rice varieties

Salt Treatment	Day (D)	Germination (N)				Total seeds (T)	Germination Energy, GE= N/T				Germination Energy Percentage, GE%				Final Germination Percentage, (FG%) 5D			
		BRRI 48	BRRI 36	BRRI 28	G-AL		BRRI 48	BRRI 36	BRRI 28	G.AL	BRRI 48	BRRI 36	BRRI 28	G.AL	BRRI 48	BRRI 36	BRRI 28	G.AL
Mock	2	10.67	9.33	6	8	100	0.1067	0.0933	0.06	0.08	10.67	9.33	6	8				
	3	38.67	28.67	26	22		0.3867	0.2867	0.26	0.22	38.67	28.67	26	22				
	4	60	44	42.67	36.67		0.6	0.44	0.4267	0.3667	60	44	42.67	36.67				
	5	75.34	63.34	58	52.67		0.7534	0.6334	0.58	0.5267	75.34	63.34	58	52.67	75.34	63.34	58	52.67
100 mM salt	2	2.67	2	0.67	2		0.0267	0.02	0.0067	0.02	2.67	2	0.67	2				
	3	20	17.34	15.34	10.67		0.2	0.1734	0.1534	0.1067	20	17.34	15.34	10.67				
	4	27.33	26	22.67	18.67		0.2734	0.26	0.2267	0.1867	27.33	26	22.67	18.67				
	5	34.67	34	33.34	28.67		0.3467	0.34	0.3334	0.2867	34.67	34	33.34	28.67	34.67	34	33.34	28.67
150 mM salt	2	1.34	0.67	0	0.67		0.0134	0.0067	0	0.0067	1.34	0.67	0	0.67				
	3	10	8.67	7.34	6.67		0.1	0.0867	0.0734	0.0667	10	8.67	7.34	6.67				
	4	16.67	14.67	14	12.67		0.1667	0.1467	0.14	0.1267	16.67	14.67	14	12.67				
	5	24	22.67	20.67	20		0.24	0.2267	0.2067	0.2	24	22.67	20.67	20	24	22.67	20.67	20

Note: Rice seeds were treated with 100 or 150 mM NaCl for 2, 3, 4, and 5 d. Results are mean values of three replicates, G.AL=GHORIAL.

3.2 Impact of salinity on root and shoot length

Root and shoot length of four rice varieties were declined in saline condition compared to control Condition (**Figure 1**). However, at 100 mM and 150mM salt treatment, the shoot and root length of BRRI 28 showed better performance followed by BRRI 48, BRRI 36 and the least performance was observed on GHORIAL (**Figure 1**). Root length of BRRI 48 was significantly decreased by 17 and 34%, BRRI 37 was decreased by 20 and 26%, BRRI 28 was decreased by 9 and 24%, and GHORIAL was decreased by 38 and 41% of 100 mM and 150 mM salt treatment respectively (**Figure 1A**). While, shoot length of BRRI 48 was 27 and 43%, BRRI 37 was 19 and 21%, BRRI 28 was 22 And 42%, and GHORIAL was decreased by 9 and 17% of 100 mM and 150 mM salt treatment respectively (**Figure 1B**). Decline of seedling height is a familiar aspect of many yield plants grown under salinity stress [19]. The gradual reduce the length of root with the increment in salinity due to higher inhibitory impact of salt stress to root expansion compared to shoot expansion [20]. The data suggest that plant root and shoot were decreased in saline condition.

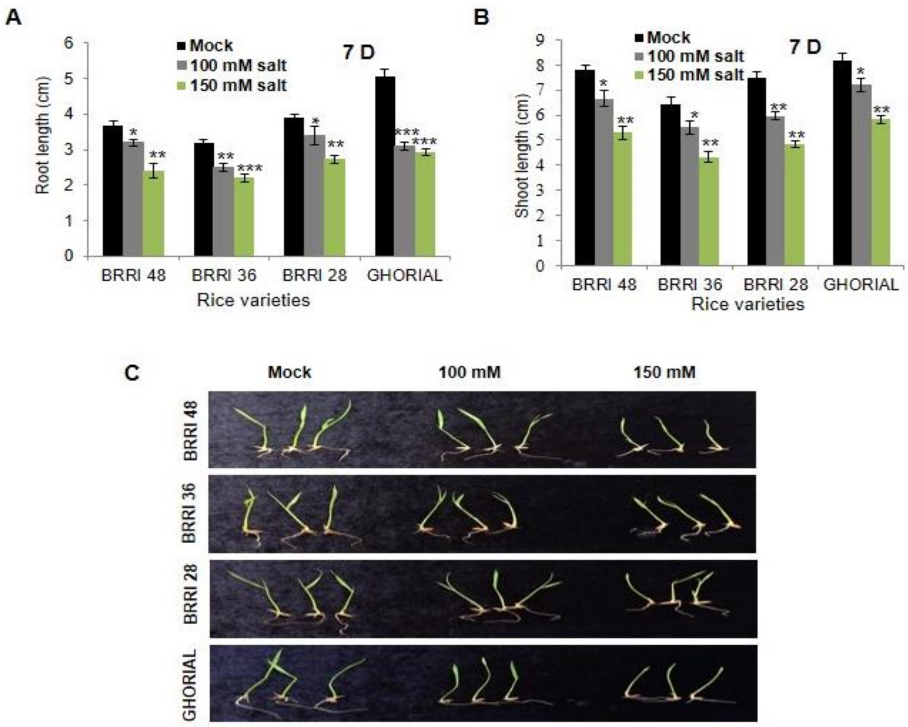

Figure 1 Effect of salinity on rice seeds germination.
Rice seeds (A to C) were treated with 100 and 150 mM salts for 7 D. Root (A) and shoot (B) were measured, and the morphology (C) of salt treated rice seedling. Data are mean of one experiment with three repeat. *, ** and *** indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

3.3 Impact of salinity on the dry weight of rice varieties

Rice seedlings dry weight were oppositely proportional to salt concentration. When the salt concentration increased then dry weight of plant seedlings were decreased. On an average, the level of 100 mM and 150 mM salt treatment, seedlings dry weight were decreased, when compared to control seedlings (**Figure 2A**)**.** Dry weight of BRRI 48 seedlings were declined by 20 and 30% of 100 mM and 150 mM salt treatment with compared to control seedlings at 7 days respectively (**Figure 2A**). However, dry weight of BRRI 37 seedlings were

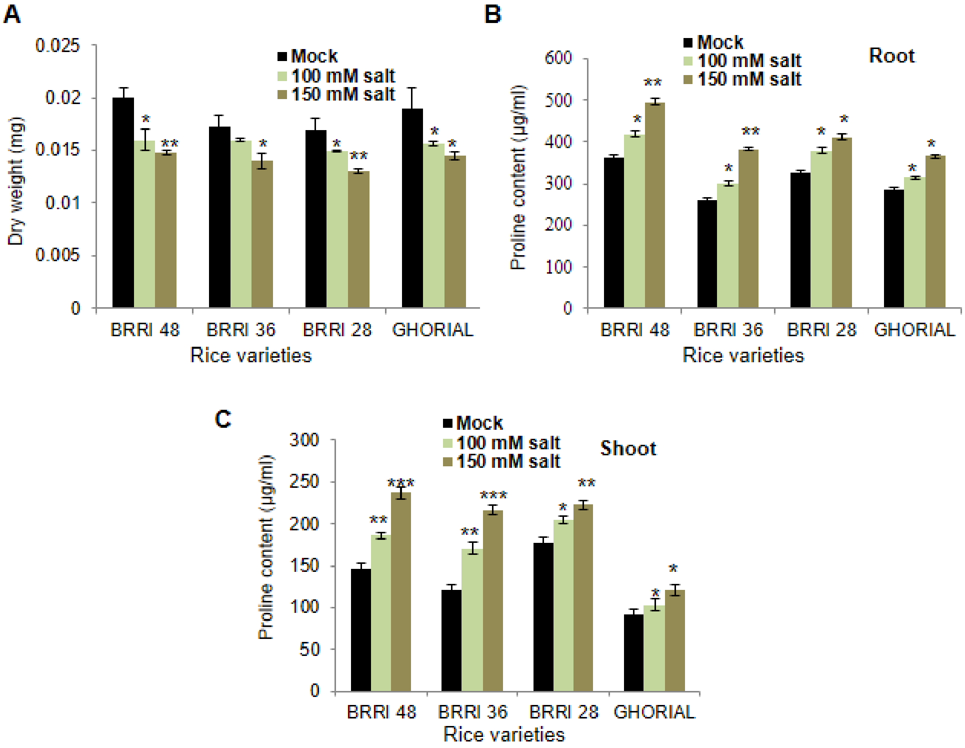

Figure 2 Effect of salinity on proline content on rice seeds germination.
Rice seeds were treated with 100 and 150 mM salts. Dry weight (A), proline content in root (B) and shoot (C) were measured. Data are mean of one experiment with three repeat. *, ** and *** indicate significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

declined by 7 and 19%, BRRI 28 was 12 and 23%, and GHORIAL was 17 and 23% of 100 mM and 150 mM salt treatment at d days respectively (**Figure 2A**). Jamil and Rha [21] found that the length of seedlings and the dry weight of various varieties were decreased as salt concentration increased. This confirms the previous findings which indicated that salt stress retarded of tomato, pea and rice [22], although shoot dry weight was more conscious to salt stress compared to root dry weight. The retardation in plant seedlings growth may be due to adverse effects of the NaCl as well as irregular nutrient elevation by the seedlings. This also approves the reports of high salt concentration could stifle growth of root and shoot due to slowing down the water uptake by the plant suggested that salinity can inhibit the root and shoot elongation and also its capacity to uptake water and essential mineral nutrient from water [23]. It is indicated that salinity decreases rice dry weight.

3.4 Impact of salinity on proline content

Similar responses had been found within this present research and salinity stress resulted into a pointy growth in proline content material. However, the fee of salt pressure-brought about proline accumulation become substantially higher. In control plants, the proline content became almost similar, however, the charge of proline accumulation turned into found plenty higher at 150 mM as compared to control (**Figure 2B, 2C**). A well-known osmo-protectant, proline is crucial for maintaining osmotic balance, protecting subcellular structures and enzymes, and raising cellular osmolarity, which gives cells the turgor they need to expand when under stress. The result indicate that the rate of proline accumulation was observed much higher at 150 mM salinity stress as compared with control.

3.5 Impact of putrescine on seed germination

Although salt treatment decrease rice seeds germination (Table 2), length of root and shoot (Figure1), Putrescine (Put) can play distinct role for this event. We treated rice seeds with 5 mM Put of 150 mM salt treatment or without salt treatment at 5 days. Rice seeds germination, shoot and root length were significantly increased by 11, 46, and 29% with 5 mM Put of 150 mM salt treatment, when compared to 150 mM salt stress (**Figure 3A, 3B and 3C**). While, seeds germination, length of shoot and root were substantially increased by 12, 37, and 32% with 5 mM Put without salt treatment, when compared to control conditions (**Figure 3A, 3B and 3C**). The physiological changes caused by salinity stress are tremendous after long term salt stress, which eventually affects plant growth [24]. PAs have been shown playing a critical role in plant salt tolerance [25], where PAs were either expanded or reduced by salt treatments. These result suggest that Put enhances rice seeds salt tolerance.

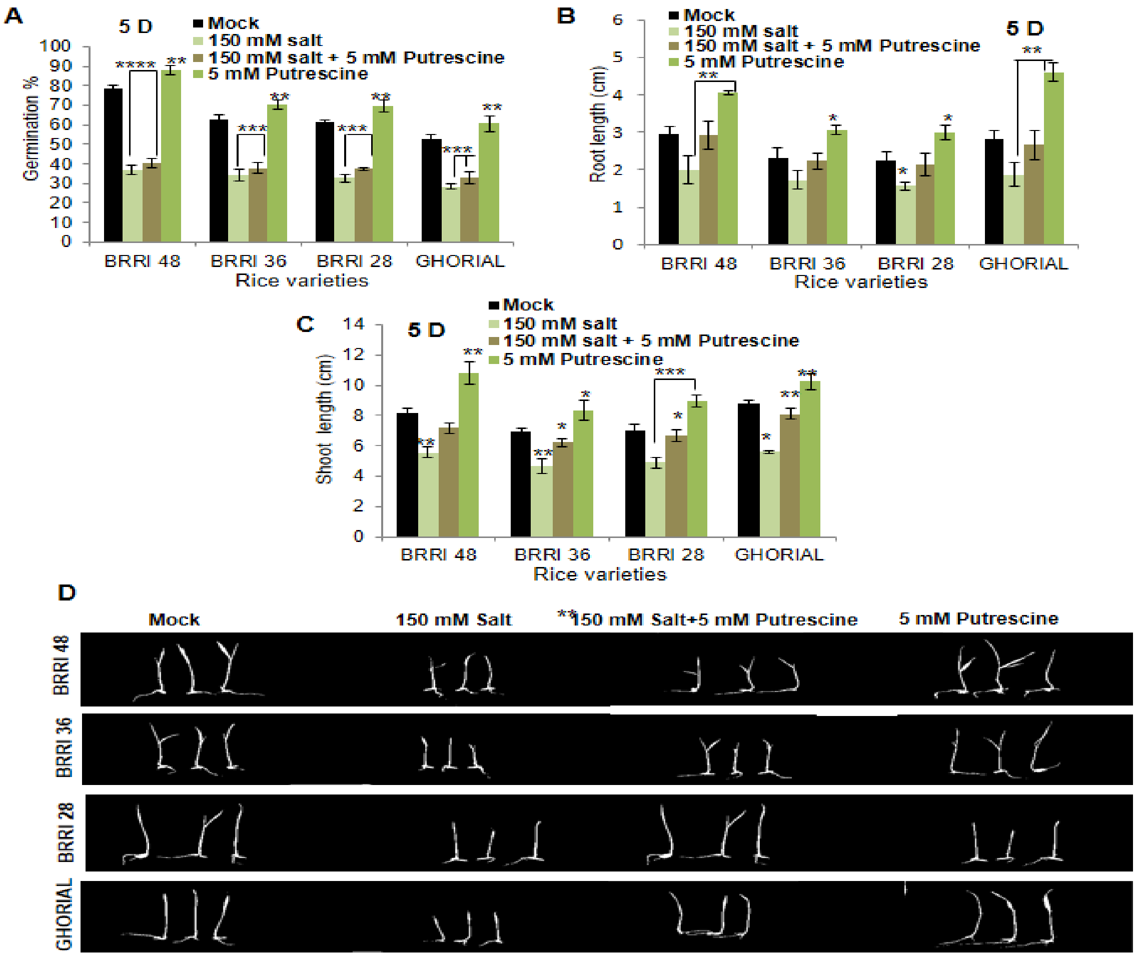

Figure 3 Effect of putrescine on rice seeds germination.

Rice seeds were treated with 150 mM salts and 5 mM putrescine. Seeds germination (A), root (B) and shoot length (C), and the morphology (D) of salt and putrescine treated rice seedling were measured. Data are mean of one experiment with three repeat. *, **, *** and **** indicate significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$ respectively.

4. CONCLUSION

Germination is a complicated phenomenon regarding many physiological and biochemical changes that result in the activation of embryo. Salinity ends in a lower inside the boom of the vegetation and decrease inside the yield and excellent of the crops. However, it has been found that salinity adversely affected the germination, shoot and root length of rice, while putrescine enhanced rice seeds germination as well as shoot and root elongation.

5. ACKNOWLEDGEMENT

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