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Ameliorative effects of biological treatments on growth of squash plants under salt stress

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Quantum Growth® was formerly Inoculaid

Abstract

The objective of this work was to evaluate the effect of selected biologicals on direct seeded and transplanted squash plant growth and mineral content under salinity stress. The study was conducted in pot experiments using a mixture of sandy loam soil:vermiculite (1:1, v:v) under controlled greenhouse conditions. Biologicals tested included AgBlend, SoilBuilder, Yield Shield, PlantShield, Inoculaid and Equity. Salinity treatments were established by adding 0, 50 and 100 mM of NaCl to a base complete nutrient solution (Hydro-Sol + $Ca(NO_3)_2$). Pots were irrigated with NaCl solutions and biological treatments were included in the water. Yield Shield was applied as a seed treatment. Salinity negatively affected growth of squash; however, biological treatments significantly increased fresh weight compared to non-treated plants that were challenged with salt stress. Furthermore, biological treatments tested increased the uptake of potassium compared to the non-treated control in both direct seeded and transplanted squash. Sodium concentration was not affected by biologicals in directed seeded squash except for SoilBuilder, Yield Shield and Equity at 100 mM, while AgBlend, SoilBuilder, Inoculaid and Equity decreased sodium uptake in transplants under salt stress. The most effective biologicals increased the K^+/Na^+ ratio, which was positively correlated with plant growth. Alteration of mineral uptake may be one mechanism for the alleviation of salt stress. Based on the results of the experiment reported herein, the use of biological treatments may provide a means of facilitating plant growth under salt stress.
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Keywords: Squash; Biological treatments; Salt stress

1. Introduction

High concentrations of salts in soils account for large decreases in yield of a wide variety of crops all over the world. Globally, more than 770,000 km^2 of land is salt-affected by secondary salinization: 20% of irrigated land, and about 2% of dryland agricultural land (FAO, 2000). Squash is an important vegetable crop for human nutrition in the world, and squash plant growth was shown to be moderately sensitive or moderately tolerant to salinity depending on cultivar or growth stage (Francois, 1985).

Salt stress affects many aspects of plant metabolism and, as a result, growth and yields are reduced. Excess salt in the soil solution may adversely affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. Specific ion effects may cause direct toxicity or, alternatively, the insolubility or competitive absorption of ions may affect the plant's nutritional balance (Greenway and Munns, 1980). Salinity was shown to increase the uptake of Na^+ or decrease the uptake of Ca^{2+} and K^+ (Neel et al., 2002).

Plant growth-promoting rhizobacteria (PGPR) and fungi can facilitate plant growth indirectly by reducing plant pathogens, or directly by facilitating the uptake of nutrients from the environment, by influencing phytohormone production (e.g. auxin, cytokinin, or giberallin), and/or by enzymatic lowering of plant ethylene levels (Björkman et al., 1998; Grichko and Glick, 2001). In addition to facilitating the growth of plant, plant growth-promoting microorganisms can protect plants from the deleterious effects of some environmental stresses including flooding (Grichko and Glick, 2001), drought (Mayak et al., 2004a), salt (Mayak et al., 2004b) and

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The microbes in Quantum Growth help plants, trees and turf recover from salt damage and grow better in soils with high salt concentrations.

phytopathogens (Harman and Björkman, 1998). In the present study, selected biological treatments were evaluated to increase squash growth under saline conditions from direct seeded and transplanted squash.

2. Material and methods

Experiments were conducted in controlled greenhouse conditions, and plants were maintained at a day/night temperature of 24/21 °C with 14 h photoperiod during the day time at Cornell University's New York State Agricultural Experiment Station (NYSAES), Geneva in 2004 and 2005. Squash (*Cucurbita pepo* L. zucchini 'Grey') was used as plant material. The initial germination of the seed lot was 97%, and seedling emergence was uniform in all treatments.

Salinity treatments were established by adding 0, 50 and 100 mM of NaCl to a base complete nutrient solution (Hydro-Sol + $Ca(NO_3)_2$). The composition of the Hydro-Sol (Peters Fertilizers, W.R. Grace & Co., Fogelsville, PA, USA) was (ppm): N, 50; P, 48; K, 210; Mg, 30; SO_4, 117; Na, 3.619; Cl, 0.040; Fe, 3.0; Zn, 0.15; Cu, 0.15; B, 0.5; Mn, 0.5; Mo, 0.1. The solution was prepared by adding 1 g Hydro-Sol and 0.66 g $Ca(NO_3)_2$ per liter of distilled water. The electrical conductivities as well as the osmotic potentials of these solutions after adding 0, 50 and 100 mM of NaCl were determined with a conductivity meter, Basic Conductivity meter (Cole-Parmer Instrument Company) and an osmometer, Osmette Model 5004 (Precision Systems Inc.). Electrical conductivity (EC) and osmotic potential of these solutions were 1.91 dS m^{-1} with −0.0004 MPa for 0 mM NaCl, 7.03 dS m^{-1} with −0.23 MPa for 50 mM NaCl, and 11.9 dS m^{-1} with −0.45 MPa for 100 mM NaCl.

2.1. Direct seed experiment

Seeds were sown in plastic pots (10 and 7 cm top and bottom diameters, respectively, and 9-cm height, with holes in the bottom). Five seeds were sown 3 cm deep per pot, filled with a mixture of Arkport sandy loam soil:vermiculite (1:1, v:v). Moisture content of this soil medium was about 14%. Soil moisture content was increased to 60% of its water holding capacity with all biologicals (Table 1), mixed in solutions at recommended dosages by manufacturer before sowing except Yield Shield. Yield Shield was applied as a seed treatment. All pots were randomized on benches in the greenhouse. After planting, pots were covered with transparent plastic to reduce evaporation until emergence beginning. All pots were irrigated to field capacity with 0, 50 or 100 mM saline solutions to maintain the level of salinity after emergence whenever soil water content reached 70% of the available water. The pots from one replication of all treatments were weighed every day to determine when to irrigate. In the study seedling emergence was uniform in all treatments, which was statistically not important (data not shown). Individual plants were harvested from above the ground 21 days after sowing and their fresh weights determined.

2.2. Transplant experiment

Seeds of squash were planted in 128-cell Styrofoam trays (Speedling, Sun City, FL, USA) in 'Cornell Mix' (peat moss, 0.28 m^3; vermiculite, 0.34 m^3; dolomitic limestone, 4.54 kg; 10–5–10 fertilizer, 1.36 kg) with one seed per cell on 15 December 2004. During the sowing and emergence, until the transplanting stage, pots were watered with the solution prepared by adding 1 g Hydro-Sol and 0.66 g $Ca(NO_3)_2$ per liter of distilled water. Enough sized, healthy and homogeneous two squash seedlings were transplanted to plastic pots (13 and 10 cm top and bottom diameters, respectively, and 15-cm height, with holes in the bottom) with a mixture of Arkport sandy loam soil:vermiculite (1:1, v:v) on 27 December 2004. After transplanting, plants were irrigated with solutions mentioned above by adding biologicals, except Yield Shield (Table 1) at recommended dosages by the manufacturer. In the first irrigation NaCl was added to the nutrient solution at ratios of 0, 25 or 50 mM. Then 0, 50 or 100 mM of NaCl concentrations were added to solutions in later irrigations. All pots were randomized on the benches in the greenhouse. All pots were irrigated to field capacity with 0, 50 or 100 mM saline solutions to maintain the level of salinity after emergence whenever soil water content reached 70% of the available water. The pots from one replication of all treatments were weighed every day to determine when to irrigate. Plants were harvested from above the ground on 16 January 2005 and their fresh weights determined.

Table 1
Biologicals used in the study and their sources

Biologicals	Ingredients	Sources
SuperBio®AgBlend™	*Bacillus* species, microbial by-products	Advanced Microbial Solutions, LLC, 801 Hwy 377 South, PO Box 519, Pilot Point, TX 76258, USA
SuperBio®SoilBuilder™	*Bacillus* species, actinomycetes, cyanobacteria, algae, protozoa, and microbial by-products	Advanced Microbial Solutions, LLC, 801 Hwy 377 South, PO Box 519, Pilot Point, TX 76258, USA
Yield Shield	*Bacillus pumilis* GB34	Bayer CropScience 2 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA
PlantShield HC	*Trichoderma harziannum*	BioWorks Inc., 51 Central Ave., Geneva, New York 14456, USA
Inoculaid	Photosynthetic bacteria	Applied and Experimental Microbiology, 7035 Phillips Highway Suite # 5-108, Jacksonville, FL 32216, USA
Equity	*Bacillus subtilis*, *Bacillus licheniformis*, *Paenibacillus polymyxa*, *Paenibacillus azotoformans*	Naturize BioSciences LLC, 11760 Marco Beach Drive, Suite 1, Jacksonville, FL 32224, USA

2.3. Mineral analysis

The oven dried leaf tissues for both direct seeded and transplanted experiments were ground to fine powder. Aqueous plant digest solutions (ca. 1–4% HNO_3) were analyzed for sodium, potassium and calcium on a Perkin–Elmer (P–E) Model 305B Atomic Absorption Spectrophotometer employing a 4-in. (10 cm) flame path of acetylene–air in the oxidation mode.

2.4. Statistical analysis

The statistical analysis was conducted using the GLM procedure of SAS (SAS, 1985). Experimental design was hierarchical with respect to two factors arranged in a completely randomized design with four replications. The first factor (NaCl levels) had three levels (0, 50 and 100 mM), and the second one had seven levels for direct seed experiment or six levels for transplant experiment (different biological treatments) (3×6 and 3×7 factorial experimental design). Data were subjected to analysis of variance (ANOVA) to compare the effects of salt stress treatments and different biological treatments. Data from each treatment was then compared with the control treatment using Dunnett's procedure at $p < 0.05$. Simple correlation analyses were performed to indicate possible relationships between the parameters analyzed.

3. Results

3.1. Direct seed experiment

3.1.1. Fresh weight

In the study a high quality seed lot was used with 97% germination to ensure the effect of biological treatments. There were no significant differences between treatments in regard to seedling emergence (data not shown).

External NaCl salinity up to 100 mM decreased weight of squash plants. Pots treated with biologicals, except for PlantShield and Inoculaid at 0 mM, Inoculaid and Equity at 50 mM, and Equity at 100 mM showed greater growth than non-treated plants. The greatest mean fresh weight was obtained by Yield Shield seed treatment in all NaCl concentrations (Table 2).

Table 2
Direct seeded squash plant fresh weight (g) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	6.13	3.40	1.57
AgBlend	6.34*	3.53*	1.66*
SoilBuilder	6.53*	3.71*	1.74*
Yield Shield	6.80*	3.89*	1.93*
PlantShield	6.05	3.71*	1.81*
Inoculaid	6.08	3.33	1.75*
Equity	6.25*	3.41	1.61

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Table 3
Direct seeded squash leaf K^+ concentration (%) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	4.51	3.88	3.19
AgBlend	4.67*	4.43*	3.64*
SoilBuilder	4.73*	4.35*	3.68*
Yield Shield	4.90*	4.38*	4.15*
PlantShield	4.55	4.18*	3.17
Inoculaid	4.53	3.85	3.80*
Equity	4.75*	3.88	3.73*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

3.1.2. Mineral content

Leaf K^+ concentration decreased as salinity increased. All biologicals except for PlantShield and Inoculaid increased K^+ compared to the control in absence of salinity. Inoculaid and Equity had no effect on K^+ content in 50 mM NaCl, while biologicals except for PlantShield caused the increased K^+ content compared to the control in the squash plants at 100 mM. Plants from seeds treated with Yield Shield had the highest K^+ content at 100 mM (Table 3).

Salinity increased Na^+ in leaves of squash, while biological treatments had no effect on Na^+ content in 0 and 50 mM NaCl. There were significant differences between control and SoilBuilder, Yield Shield and Equity in regard to Na^+ content in 100 mM NaCl (Table 4).

Salinity decreased Ca^{2+} content, while biological treatments except for PlantShield at 0 mM, AgBlend and PlantShield at 50 mM, and AgBlend, SoilBuilder and PlantShield at 100 mM increased Ca^{2+} when compared to control (Table 5).

The K^+/Na^+ ratio was significantly decreased with the increasing salinity stress. There were no statistically differences between treatments at 0 mM of NaCl. AgBlend, Yield Shield and SoilBuilder had the greater K^+/Na^+ ratio than control treatment in 50 mM NaCl. PlantShield was the only treatment which did not increase K^+/Na^+ compared to control in 100 mM NaCl (Table 6).

Table 4
Direct seeded squash leaf Na^+ concentration (%) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	0.23	0.28	0.36
AgBlend	0.23	0.28	0.34
SoilBuilder	0.25	0.29	0.31*
Yield Shield	0.25	0.29	0.31*
PlantShield	0.26	0.29	0.35
Inoculaid	0.26	0.28	0.34
Equity	0.25	0.26	0.33*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Table 5
Direct seeded squash leaf Ca^{2+} concentration (%) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	1.38	1.17	0.73
AgBlend	1.62*	1.23	0.79
SoilBuilder	1.53*	1.60*	0.73
Yield Shield	1.67*	1.39*	0.83*
PlantShield	1.36	1.24	0.65
Inoculaid	1.47*	1.52*	0.94*
Equity	1.50*	1.33*	0.93*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Table 7
Transplanted squash plant fresh weight (g) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	20.60	11.83	6.68
AgBlend	28.73*	14.55*	9.90*
SoilBuilder	24.00*	13.47*	7.85*
PlantShield	23.63	13.13*	7.55*
Inoculaid	25.10*	13.28*	9.65*
Equity	24.65*	12.98*	9.10*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

3.2. Transplant experiment

3.2.1. Fresh weight

In transplant experiment, fresh plant weight decreased dramatically with the increasing NaCl concentration. Plants treated with AgBlend, SoilBuilder, Inoculaid and Equity in 0 mM NaCl; AgBlend, SoilBuilder, PlantShield, Inoculaid and Equity in 50 mM and 100 mM NaCl displayed higher fresh weight than non-treated plants. The greatest fresh plant weight of squash was obtained with the AgBlend treatment in all range of salinity treatments. The Yield Shield treatment was not tested in the transplant experiment (Table 7).

3.2.2. Mineral content

Similar to fresh plant weight of squash transplants, K^+ decreased with the increasing NaCl concentration. All biologicals except for SoilBuilder increased K^+ content compared to non-treated plants in absence of salinity. The highest concentrations of K^+ accumulated in leaves in the presence of AgBlend under salt stress (Table 8).

Na^+ content was significantly affected by salinity and biological treatments. Salt stress increased the concentration of Na^+ in leaves of squash. Biologicals had no significant effect on Na^+ content in 0 mM NaCl. AgBlend, SoilBuilder and Equity treatments had lower Na^+ content than the control in 50 mM. Furthermore, all biological treatments used in the study except for PlantShield caused to the decreased Na^+ content of squash in 100 mM (Table 9).

Increasing the concentrations of NaCl from 0 to 100 mM lowered Ca^{2+} content in squash plants; however, Inoculaid was the unique treatment which gave the increased Ca^{2+} content compared to control plants in 50 mM NaCl (Table 10).

The ratio of K^+/Na^+ was significantly influenced by salinity and biological treatments. Increasing salinity level decreased the ratio of K^+/Na^+. There were no significant differences between treatments under no salinity. However, all biological treatments except for PlantShield increased the ratio of K^+/Na^+ under salt stress. Maximum K^+/Na^+ ratio was observed with the application of SoilBuilder at 50 mM and Inoculaid at 100 mM NaCl (Table 11).

4. Discussion and conclusion

Saline soils and saline irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth (Shannon and Grieve, 1999). The present study demonstrates salinity adversely affected the growth of squash regardless of biological treatments. However, some biological treatments off-set the negative impact of salinity on growth of squash. Plant growth-promoting bacteria were tested on growth of tomato, pepper, canola, bean and lettuce under salt stress, and these biological treatments ameliorated the deleterious effect of salinity (Glick et al., 1997;

Table 6
Direct seeded squash leaf K^+/Na^+ ratio affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	20.06	14.15	8.98
AgBlend	19.50	16.13*	10.87*
SoilBuilder	19.29	15.16*	12.15*
Yield Shield	20.42	15.49*	12.22*
PlantShield	18.00	14.65	9.28
Inoculaid	17.60	13.92	11.35*
Equity	19.02	14.95	11.34*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Table 8
Transplanted squash leaf K^+ concentration (%) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	3.66	2.98	2.28
AgBlend	3.88*	3.48*	3.05*
SoilBuilder	3.70	3.29*	2.68*
PlantShield	4.15*	3.05	2.74*
Inoculaid	4.19*	3.33*	2.90*
Equity	4.03*	3.09	2.83*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Table 9
Transplanted squash leaf Na^+ concentration (%) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	0.28	0.34	0.40
AgBlend	0.29	0.30*	0.36*
SoilBuilder	0.27	0.28*	0.37*
PlantShield	0.27	0.34	0.39
Inoculaid	0.28	0.32	0.34*
Equity	0.29	0.31*	0.36*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Table 11
Transplanted squash leaf K^+/Na^+ ratio affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	13.07	8.68	5.78
AgBlend	13.52	11.60*	8.49*
SoilBuilder	13.97	11.97*	7.28*
PlantShield	15.25	9.08	7.07*
Inoculaid	15.03	10.51*	8.53*
Equity	14.14	10.19*	7.91*

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Mayak et al., 2004b; Yildirim and Taylor, 2005; Barassi et al., 2006). Studies indicated that these positive effects might be attributed to increased water use efficiency, stimulation of root growth by production of phytohormones and/or enzymatic lowering of plant ethylene concentrations.

In this study, pots treated with biologicals, except for Inoculaid and Equity, showed greater growth than non-treated plants in the direct seeded experiment, while all biologicals increased fresh weight compared to non-treated plants in the transplant experiment under saline conditions. In the direct seed experiment, the greatest mean fresh plant weight was obtained by Yield Shield as seed treatment (Tables 2 and 7). Glick et al. (1997) have reported that when the bacterium is added directly to the seed it has an early effect on root and shoot growth. Barassi et al. (2006) showed that lettuce plants grown from plant growth-promoting bacteria inoculated seeds displayed higher total fresh and dry weights than non-inoculated control at 80 mM NaCl. The maximum fresh weight was observed with AgBlend in the transplant experiment under both saline and non-saline conditions (Table 7). In both seeded and transplant experiments all biologicals except for PlantShield and Equity increased the K^+ content in leaves of squash under salt stress (Tables 3 and 8). In the seeded experiment, Na^+ content in plants treated with SoilBuilder, Yield Shield and Equity in 100 mM NaCl was not similar to that of the control plants (Table 4), while AgBlend, SoilBuilder, Inoculaid and Equity treatments decreased Na^+ content compared to the control in transplant experiment (Table 9). All biological treatments, except for AgBlend and PlantShield increased Ca^{2+} under salt stress in seeded experiment (Table 5), whereas only Inoculaid increased the Ca^{2+} content compared to control plants in transplant experiment (Table 10). It was reported that plant growth-promoting bacteria could affect differently plant growth due to various growth stages of plant and biological treatments used. Furthermore, the way in which the plant growth-promoting bacterium is added to plant (soil or seed treatment) affects the response of the plant to the bacterium (Glick et al., 1997).

Table 10
Transplanted squash leaf Ca^{2+} concentration (%) affected by different biological treatments and salt stress

Biologicals	NaCl concentration (mM)		
	0	50	100
Non-treated (control)	1.83	1.73	1.32
AgBlend	1.86	1.80	1.31
SoilBuilder	1.83	1.69	1.35
PlantShield	1.79	1.51*	1.30
Inoculaid	1.83	1.87*	1.35
Equity	1.78	1.47*	1.31

* Means significantly different from control at $p < 0.05$ according to Dunnett's test at each salt concentration.

Salinity dominated by Na^+ and Cl^- not only reduces Ca^{2+} and K^+ availability, but reduces Ca^{2+} and K^+ transport and mobility to growing regions of the plant that affects the quality of both vegetative and reproductive organs (Grattan and Grieve, 1999). Moreover, many studies have shown that high concentrations of Na^+ and Cl^- in the soil solution may depress nutrient–ion activities and produced extreme ratios of Na^+/Ca^{2+} and Na^+/K^+ in the plants, causing the plants to be susceptible to osmotic and specific ion injury as well as to nutritional disorders that result in reduced yield and quality (Grattan and Grieve, 1999; Essa, 2002; Sivritepe et al., 2003). The results of this study showed that salinity caused an increase in Na^+ concentration and a decrease in K^+ and Ca^{2+} regardless of biological treatments (Tables 3–5 and 8–10). However, some biological treatments reduced the Na^+ uptake of plants and/or increased the K^+ uptakes compared to control treatment under salt stress, thus increasing the ratio of K^+/Na^+ (Tables 6 and 11). Significant positive correlations were determined between fresh weight and K^+ ($r = 0.901^{***}$, $r = 0.882^{***}$), Ca^{2+} ($r = 0.764^{***}$, $r = 0.841^{***}$) as well as K^+/Na^+ ($r = 0.903^{***}$, $r = 0.949^{***}$). There was a significant inverse correlation between fresh weight and Na^+ content ($r = -0.785^{***}$, $r = -0.862^{***}$) for transplant and direct seed experiments, respectively. Studies indicate that an increase in concentration of K^+ and Ca^{2+} in plants under salt stress could ameliorate the deleterious effects of salinity on growth and yield (Grattan and Grieve, 1999; Sivritepe et al., 2003). Similarly, Satti and Lopez (1994) in tomato and Kaya et al. (2003) on pepper and cucumber determined that an increase in the concentration of K^+ in the plants exposed to salt stress could ameliorate the deleterious effect of salt stress on the growth and yield. Plant growth-promoting rhizobacteria have been shown to be able to provide the plant with important minerals, e.g. nitrogen, phosphate, potassium (Singh and Singh, 1993; Altomare et al., 1999; Grichko and Glick, 2001; Egamberdiyeva and Hoflich, 2003;

Mayak et al., 2004b) in the presence or absence of salinity. Large areas of land under squash production in the world are established by sowing seed directly into the soil or transplanting. It can be interpreted from the study that seed treatment with plant growth-promoting bacteria might be suggested in case of direct seed method, while applications with irrigation water might be preferred in transplant.

The results of this study demonstrate that some biological treatments tested have a positive effect on growth of squash under salt stress. Based on these findings, the biological treatments may help alleviate the negative effect of salinity on the growth of direct seeded or transplanted squash.

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