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ORIGINAL ARTICLES

Alleviation of Adverse Effects of Salinity on Growth, and Chemical Constituents of Marigold Plants by Using Glutathione and Ascorbate

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ABSTRACT

A pot experiment was conduced at screen of National Research Centre, Cairo, Egypt during (2008-2009) to determine the effect of salinity (1500 and 3000ppm), glutathione (100 and 200 ppm) and ascorbic acid (100 and 200 ppm) on the growth and flowering behaviour of *Tagetes erecta L.* and some chemical constituents as well as essential oil percent and components. The treatments of salinity levels (1500 or 3000ppm) reduced all growth and flowering parameters (plant height, No. of branches, fresh and dry weight of herb and flowers and No. of flowers), total carbohydrates(%), total phenols, zathaphylls pigment content and mineral ions percentage). However, total indols content and essential oil percentage were increased under saline condition. Oil components percentage and amino acids were lowered by salinity except proline, glutamic and arginine. Application of glutathione or arcorbic acid (100 or 200 ppm for each) was found to be effective in increasing most of previous parameters both in saline and non-saline conditions (1500ppm). While applying glutathion or ascorbate under 3000ppm salinity had inhibitor effect.

Key words: Tagetes, salinity, glutathione, Ascorbic acid, growth, flowering, essential oil, chemical, constituents.

Introduction

Tagetes erecta L. plant family compositae (Asteraceae) with the commone name of marigold, one of the most important ornamental plants, is valued in landscape settings and also as cut flowers (Nau, 1997). Marigold flowers are rich source of a natural yellow to orange dye, helenien (a dipalmitate ester of a xanthophylls), which is in high demand by national and international companies. The flowers are used in processed food, confectionery, drugs, and pharmaceuticals and in the poultry industry (Ram *et al.*, 2000). One of the most important effects of the plant is their uses as very valuable intercrop for controlling plant parasitic nematodes and insecticidal activity was recorded by Darwish, 1992. The oil of this plant has a statistically significant antifungal and insecticidal activity (Balbaa *et al.*, 2008).

Salinity turns fertil and productive soil into barren desert and leads to alterations and loss of biodiversity of natural flora (Ghassemi *et al.*, 1995). Small changes in salt concentration are sufficient to suppress vegetative growth and plant development, thus inducing physiological changes with different direct and indirect effects (Shannon *et al.*, 1994). Salinity stops growth and, in high concentrations, changes plant morphology and anatomy and has lethal effect on plant organism (Kozlowski, 1997).

Cultivated plants cover the whole range of response to salinity from sensitivity to tolerance, including morphological, physiological and metabolic problems (Bray, 1995).

Ornamental plants are classified depending on their tolerance to salinity (Ivanova *et al.* 1990), and tagetes proved to be more tolerant (Zapryanovac and Atanassova, 2009).

Glutathione (GIT) is the most important non-protein thiol present in cell animal as well as in plants and bacteria. In plants, the physiological significances of glutathione may be divided into two categories: sulfur metabolism and defense. GIT is the predominant non-protein thiol and it is an important pool of reduced sulfur (Rennenberg, 1982) and it regulates sulfur uptake at root level (Lappartient and Touraine, 1996). In addition to its effects on expression of defense gene (Dron *et al.*, 1988 and Wingate *et al.*, 1988), GIT may also be involved in redox control of cell division (sanchez-Fernandez *et al.*, 1997). GIT plays a crucial role in controlling and maintaining the intracellular redox state (Noctor and Foyer, 1998).

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Ascorbic acid (AS) is an organic compound required in trace amount to maintain normal growth in higher plants (Podh, 1990). AS influence mitosis and cell growth in plants (Noctor and Foyer, 1998; Smirnoff and Wheeler, 2000), affects phytohrmone- mediated signaling processes during the transition from the vegetative to the reproductive phase as well as the final stage of development and senescence (Barth *et al.,* 2006). Furthmore, AS affects nutritional cycle's activity in higher plants and plays an important role in the electron transport system (Liu *et al.,* 1997). It is also important as a cofactor for a large number of key enzymes in plants (Arrigoni and de tullio, 2000).

Reduced glutathione, and reduced ascorbate, the two major water soluble antioxidants in photosynthetic and non-photosynthetic tissues, reacting directly or indirectly with reactive oxygen species, contribute to maintain the integrity of cell structures and the proper functions of various metabolic pathways (Bielawski and Joy, 1986; Conklin *et al.,* 1997).

The aim of the present study was to investigate the relationship between growth, flowering, essential oil and some chemical constituents dected in marigold as an effect of irrigation with saline water and the effect of glutathion and ascorbic acid to understand their affect on plant balance.

MATERIALS AND METHOD

A pot experimental trails were carried out during the two successive seasons of (2008 and 2009) at the screen of National Research Centre, Cairo, Egypt seeds of *Tagetes erecta* L. which were obtained from horticulture Research Institute, Agric. Res. Centre, Ministry of Agric., A.R.E. The seeds were sown in is March for both seasons in sand soil. The physiological and chemical properties of the tested soil were presented in table (1) Jackson (1967) after 45 days in both seasons, the homogenous seedling of 2-3 pairs leaves were transplanted in pots of 30 cm diameter that filled with 12 kg of soil.. All plants were supplied by super phosphate 15.5% P_2O_5 at the rate of 3.g/pot before trans planting while ammonium sulphate (20.5 %N) at the rate of 2.g/pot were added twice, the first addition was applied after transplanting and the second was after 15 days from the first. In the same time, also potassium fertilizer was applied sulphate (48%K) at the rate of 2.g/pots. The plant irrigation with saline water as (1500 and 3000ppm) sodium chloride (NaCl, Calcium chloride and sulphate. Magnesium (1: 1: 1) to near field capacity

Plants were sprayed twice with freshly prepared solutions of Glathione and ascorbic acid each at 100 and 200ppm, and combination of the different concentrations of the two factors. The experimental design was a complete randomized with three replicates, where each replicate represented by 3 pots.

Table 1: Some physical and chemical properties of the used soil

Properties	Value	Nutrient	Content	Value
		N		3.2
Sand %	3.3	P	mg/100	0.05
Silt %	0.4	K	g soil	2.5
Clay %	4.3	Mg		2.9
Texture	Sandy	Fe		15.2
Ec dsm^{-1}	2.1	Mn	ppm	3.8
pH	7.5	Zn		16.3
$CaCO_3$%	2.10	Cu		0.8

The plant herbage was harvested by cutting above 6cm over the soil surface and the following data were recorded, plant height, number of branches/plant, fresh and dry weight of herb/plant, No. of flowers and fresh and dry weight of flowers/plant.

Chemical Analysis: Total carbohydrates were determined according to Dubois *et al*. (1956). Total xanthphyils estimated by A.O.V.C (2006).

Total nitrogen was determined by micro kjeldahl methods described by markham (1942), while phosphorus determination was carried out calorimetrically according to king (1951). Potassium was determined by atomic absorption spectrophotometry using a Perkin Elmer Model 370 A.A. Spectrophotometer. The recorded data (means of the two growing seasons) were statistically analyzed according to the procedure of Sendecor and Cochran (1980), while the means of the studied treatments were compared using L.S.D test at 0.05 of probability.

Total phenols: 1gm of the fresh leaves were macerated in 5-10 ml 80% ethanol for at least 24 hr. at 0°C, the alcohol was clarified the remained residue was re-extracted with 5-10 80% ethanol 3 times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. The colorimetric method of folin-Denis as described by Daniel and George (1972). Total indoles: Total soluble indoles were determined colorimeterically according to the method described by Selim *et al*. (1978).

The essential oil determined: The oil percentage of fresh herb samples at each cut was extracted by water distillation for 6hr and then dried over anhydrous sodium sulphate and determined according to Guenther (1961). Gas liquid chromatography analysis of essential oil. The oil was injected in Perkin Elmer gas chromatography model. X1- with a split ratio 1: 10. The oil constituents were separated on 60 m DB-S capillary column having 0-32 mm interval diameter, the initial and final temperatures were 50-230°C, rate 3°C/min. Injector and detector (F.I.D) temperature were 230 and 250°C, respectively Helium was used as a carrier gas at a flow rate of 1ml/min. Identification of components was performed by GC-MS using Hp instrument. The conditions were the same as GC, the electron impact ionization was set at 70ev, while the ion source temperature was 275 Co and the scan rate was 29-400Amu/sec. constituents were also indentified by comparing their retention time with those of standard samples.

Amino acid determination: amino acids were determined in leaves according to the method described by Widner and Eggum (1966). Oxidation was carried out by using performing acid, to protect methionine and cysteine from destruction during acid hydrolysis, following acid hydrolysis in the oven at 110°C for 24 hr. High performance amino acid analyzes, Beckman 7300 was used for amino acid determination.

Results and Discussion

Plant Growth and Flowering Characters:

Results presented in table (2) reveal that 3000 ppm salinity level reduced plant hight, number of branches/plant and fresh and dry weights of herb/plant as well as flowering characters (number of flowers/plant and fresh and dry weights of flowers/plant) of *Tagetes erecta L.* as compared with control treatment. Exogenous application of 200ppm. Glutathione (GIT) or Ascobic acid (AS) as a foliar spray was found to be effective in increasing all the previous mentioned parameters significantly both in saline (1500 ppm) and non-saline conditions and this may be related to their inhibiting effect on Cl^- and Na^+ and improving the uptake of N, Mg, Fe, Mn and Cu. The reduction due to salinity attributed not only to inhibition of water absorption and ion toxicity but also to the nutrient disturbance under such conditions (Helal *et al.,* 1975), also Dunlap and Binzel (1996) mentioned that increasing NaCl concentration can reduce the endogenous level of IAA which may be critical to water movement through the root system of plants. Exgenous applications of asmoprotectents, plant growth regulators, fertilizers, and antioxidants have been reported to successfully mitigate the adverse effects of salinity on plant growth and metabolism (Shalata and Neumann, 2001). Of the various non-enzymatic antioxidants such as carotenoids, phenols and ascorbic acid occurs ubiquitously in plants and have been reported to play a vital role in alleviating the adverse effects of salt on plant growth and metabolism in many crop plants (Hamada, 1998). Hemmat (2007) found that exogenous application of glutathione mitigated partially or completely the adverse effects of salt stress on growth of *Canola* seedlings, thereby glutathione is integrated into primary metabolism and influence the functioning of signal transduction pathways by modulating cellular redox state, it may be affected nuclear gene expression which influenced by plant's external environment.

Chemical Constituents:

Total Carbohydrates (%):

The data in table 3 pointed out that saline stress (300 ppm) significantly reduced the percentage of total soluble carbohydrates in *Tagetes erecta L.* plants. A more pronounced increase in total carbohydrates was obtained with exogenous applications of 200 ppm GLT or AS as a foliar spray in salin condition (1500ppm). The change in soluble sugars content under salt stress has been already reported for a number of species (Ashraf and Tufail, 1993 and Amini and Ehsanpour, 2005). The reduction in soluble sugars may be attributed to the decline in the photosynthetic pigments or to reduction in the CO_2 assimilation rate, stomatal conductance (Downton, 1977). The interactive effects of glutathione or ascorbic acid on accumulation of soluble sugars probably, attributed to the protective effects of glutathione and ascorbic acid on the photosynthetic systems, also, plays a protective role in salinity tolerance by maintenance of the redox status (Chaparzadeh *et al.,* 2004 and Ameer *et al.,* 2006).

Total Phenols:

The total phenolics of *Tagetes erects L.* plants subjected to saline stress (1500 or 3000ppm) were significantly lower compared to that of control unstressed plants (table 3). Foliar application of GLT or AS

acid (100 and 200 ppm) stimulate the accumulation of phenols under non-saline condition. However, their application at 200 ppm significantly enhanced phenols levels under 1500 ppm salin conditions. The reduction in phenols levels under salt stress may be due to its oxidation by the antioxidant enzymes with withdraw phenols as their substrate and may also, due to the decline in its biosynthesis. Phenols protect the cells from potential oxidative damage and increase stability of cell membrane (Burguieres *et al.,* 2006).

Table 2: Effect of salinity levels, glutathione and ascorbic acid as well as their interaction on growth and flowering parameters of *Tagetes erecta* L. (mean of the two seasons).

Treatment (ppm)	Plant hight (cm)	No. of branches /plant	F.W of herb/ plant (g)	d.w of herb/ plant (g)	No. of flowers/ plant	F.W of flowers/ plant	d.w of flowers/ plants
Control	43.4	5.12	87.2	13.41	7.56	13.42	4.34
Salinity							
1500	42.2	4.14	85.10	13.24	6.21	11.45	3.09
3000	33.8	2.88	57.33	9.23	3.65	6.77	2.41
Glutathione (GIT)							
100	51.2	5.88	118.72	19.02	9.11	17.25	6.72
200	61.3	9.10	148.11	30.20	14.22	27.52	9.45
Ascorbic acid (AS)							
100	59.6	8.10	125.13	21.16	9.73	17.85	6.81
200	65.4	10.52	156.22	35.81	17.45	31.34	10.22
Interactions							
Salinity 1500 +GIT100	51.0	5.00	109.41	18.73	9.00	17.23	6.25
Salinity 1500 +AS100	59.2	7.82	122.34	20.00	9.70	17.47	7.42
Salinity 1500 +GIT200	60.0	8.76	128.37	27.81	14.21	27.11	10.52
Salinity 1500 +AS200	63.8	10.00	135.25	29.11	17.11	31.08	10.43
Salinity 300 + GIT100	38.8	3.27	80.24	12.42	5.62	10.13	3.21
Salinity 3000 +AS100	40.6	3.71	85.92	13.65	6.73	11.35	3.41
Salinity 3000 +GIT 200	41.7	4.25	85.18	13.0	8.33	14.43	4.25
Salinity 3000 +AS200	42.8	4.71	89.62	14.62	8.94	15.23	4.79
LSD (0.05)	2.54	0.898	2.896	0.041	0.095	0.113	0.008

Total Indols:

Results in table 3 indicate that total indols content in *Tagetes erecta* plant was increased under saline conditions (1500 or 3000 ppm) compared with control plants. Also the interaction between 1500 ppm salinity and GlT or AS each at 100 or 200 ppm resulted in promotive effect on total indols content while applying GLT or AS alone or combined with 3000 ppm salinity had inhibitor effect. The results in harmony with those of Nahed *et al.* (2009) on Gladiolus plants.

Zenthaphyll Pigment Content:

Salt stress had inhibit effect on the zanthophyll pigments level in the *Tagetes erects* L. plants as shown in table 3 GIT or AS at 200ppm significantly increased zanthaphyll pigment content under saline stress (1500ppm) or non saline condition to highest values compared to control plants. The ameliorating effect of glutathione and ascorbic acid on pigments level may be due to their effects on either enhancing the photosynthetic activities or retardation of chlorophyll degradation resulted from the oxidative stress (Hammat, 2007).

Oil Percentage:

Data is table 3 reveal that oil percentage is in harmony with zanthaphyll pigment content, due to salinity, GIT and AS levels. Salinity at 3000 ppm level increased oil %, compared to the control. Applying 100 or 200 ppm GIT or AS as foliar application under 1500ppm salin or non saline conditions significantly increased oil % to highest values. In this concern, El-Shafey *et al.* (1991) reported that oil percentage of sweet basil increased at the lower salinity levels.

Mineral Ions Percentage:

From the results presented in table 3, it is evident that foliar application of GIT or AS (100 or 200 ppm) under non saline-conditions significantly increased N, P and K percentage in *Tagetes erects* L. plants compared with control. While high water salinity level (3000 ppm) led to decreasing of N, P and K% to lowest ratios. The results go in line with those of Talaat and Aziz (2005) on chamomile and El-Gabas (2006) on sunflower

plants who mentioned that glutothione and ascorbic acid significantly increased macroelements content.

Increasing water salinity levels led to increase in Na, Cl and Ca% but decrease N and K% as well as potassium/sodium ratio. These accumulation of sodium, calcium and chlorine in plant tissues might mean that salinity is linked to its limited efficiency in keeping Na and Cl in leaf tissue below toxic levels and compensating for the lower water potentials associated with salinity by increasing tissue levels of organic solutes (Rush and Epstein, 1976).

Oil Constituents:

GC/ MS analysis shown in Table 4 in indicates that the indentified compounds in the oil of *Tagetes erecta L.* treated and control plant mainly consisted from cis-b-ocimene (0.11-0.85%), followed by Beta – Farnesene (0.46-2.11%), Trans- linaloal oxide (0.11-0.88%), Pepretone (1.26-10.24%), Beta-Jonone (8.78-20.45%), Trans caryophyllene (15.26 - 30.41%), caryophyllene oxide (2.18- 8.80%), eugenol (11.21- 33.42%) linalool (1.87-5.22%) and Hexa-decanoic acid (0.21-2.83%). This finding coincided with those of Machado *et al.* (1994) on the components of *Tagetes erecta L* leaf oil.

Table 3: Effect of salinity levels, glutathione, ascorbic acid and their interactions on chemical constitutents, essential oil content and macronutrients of *Tagetes erecta L.* plants (mean of the two seasons)

Treatment (ppm)	Total carbohy-drates (%)	Total phenols	Total Indols	Zantho-phylls	essential oil (%)	Macronutrients (%)		
		mg/g Fw				N	P	K
Control	20.3	3.28	8.94	0.10	0.22	3.28	0.62	6.71
Salinity								
1500	20.1	2.19	9.43	0.8	0.22	3.22	0.42	5.23
3000	16.2	1.08	10.21	0.7	0.25	2.12	0.21	3.11
Glutathione (GIT)								
100	21.5	3.74	7.22	0.18	0.25	4.18	0.67	6.83
200	23.4	4.18	8.11	0.27	0.30	4.62	0.74	7.43
Ascorbic acid (AS)								
100	22.2	4.16	6.87	0.13	0.23	4.27	0.69	6.95
200	27.5	4.29	8.00	0.25	0.28	4.68	0.79	7.68
Interactions								
Salinity 1500 +GIT100	21.5	2.38	10.23	0.21	0.21	4.01	0.41	5.64
Salinity 1500 +AS100	22.8	2.52	10.11	0.18	0.19	4.23	0.47	5.71
Salinity 1500 +GIT200	28.5	3.14	10.45	0.23	0.23	4.51	0.44	6.34
Salinity 1500 +AS200	28.9	3.28	10.23	0.23	0.21	4.53	0.48	6.72
Salinity 300 + GIT100	17.3	1.89	7.41	0.19	0.16	3.01	0.22	4.11
Salinity 3000 +AS100	17.8	1.92	7.01	0.18	0.15	2.23	0.24	4.21
Salinity 3000 +GIT 200	13.4	1.23	5.23	0.12	0.18	2.11	0.19	2.08
Salinity 3000 +AS200	13.2	1.42	4.35	0.10	0.16	2.08	0.16	2.13
LSD (0.05)	0.02	0.038	0.213	0.003	0.001	0.021	0.004	0.03

It is clear from data tabulated in table 4 that salinity level (1500 or 3000 ppm) decreased those components percentage compared to their percentage in the oil of the control. While, under GIT or AS (100 or 200ppm) treatments, the oil components percentage of *Tagetes erecta* were increased. These results were in agreement with those obtained by Youssef and Iman (2003) on *Rosmarinus officinals* L. who-found that oil composition responded greatly to foliar spray of the vitamine nicotineamide and ascorbic at different rates of application.

Table 4: GC-MS identified constituents in the essential oil of *Tagetes erecta L.* as affected by salinity, Glutathione and Ascorbic acid.

Order	Retention time	Treatment Constituents	Control	Salinity		Glutathione		Ascorbic	
				1500	3000	100	200	100	200
1	19.15	Cis-b-ocimen	0.42	0.23	0.11	0.53	0.85	0.52	0.62
2	22.74	Beta-Farnesene	1.35	0.84	0.46	1.89	2.11	1.54	1.85
3	25.62	Trans-Linalool oxide	0.53	0.31	0.11	0.84	0.88	0.62	0.69
4	28.74	Pipretone	5.24	3.23	1.26	8.71	10.24	7.21	7.32
5	35.43	Beta-Jonone	14.74	11.21	8.78	16.73	20.45	15.22	16.11
6	37.42	Trans caryophyllene	26.23	21.43	15.26	28.45	30.41	23.43	25.11
7	39.21	Caryophyllene oxide	3.27	3.11	2.18	6.79	8.80	5.17	5.87
8	40.23	Eugenol	24.21	16.45	11.21	30.23	33.42	26.41	28.22
9	41.08	Linallool	3.78	2.56	1.87	4.11	5.22	3.83	4.10
10	46.23	Hexa-decanoic acid, methyl ester	1.46	0.57	0.21	1.67	2.83	1.89	2.22

Amino Acids:

The amino acids as percentage were illustrated in table 4. All amino acids were lowered by salinity except proline, glutamic and arginine. Salinity level at 3000 ppm caused a depression which exceeded that obtained at 1500 ppm salt concentration treatment. On the contrary, proline, glutamic and arginine increased to highest ratios (5.26, 4.89 and 2.42%, respectively) under high saline condition (300ppm), compared to that of the control. Deficiency of K^+ induced by salinity increased the level of free amino acids especially of proline, aspartic and glutamic acids (Causido *et al.*, 1987). Salts stressed resulted in elevation of amino acid levels in plants (El-Bassiouny and Bakheta, 2005).

Data in table 4 indicate that all amino acids were increased with treatments of 100 or 200 ppm GIT or AS under non saline condition compared with control except hisidine, cystine and phynyl alanine. However, under 1500 ppm of saline condition, the treatment of 200ppm GIT caused increasing of Aspartic while 100 or 200 ppm GIT or AS under 1500 or 3000 ppm salinity increased proline, glutamic and arginine and lowered all other amino acids percentage. In this respect, Bezrukva *et al.* (2001) revealed that the presence of vitamins during water stress increased proline and other free amino acids in shoots of wheat plants. Furthermore, glutathione significantly enhanced the stimulatory role of slat stress on the production of free amino acid in cv. Serw green tops as well as in cv. Pactol green plants. The accumulation in amino acids in plants exposed to stress probably attributed to the disturbance is amino acid metabolism (Hemmat, 2007).

Table 5: Effect of salinity levels, glutathione, ascorbic acid and their interaction on the proportion of different amino acids in *Tagetes eracta L.* plants (mean of two seasons).

Amino acid (%)	Control	Salinity (ppm)		Glutathion (ppm)		Ascorbic (ppm)		Salinity 1500 (ppm)				Salinity 3000 (ppm)			
		1500	3000	100	200	100	200	GIT 100	GIT 200	AS 100	AS 200	GIT 100	GIT 200	AS 100	AS 200
Aspartic	1.74	1.62	0.94	1.87	2.35	1.82	2.22	1.74	2.11	1.72	2.10	0.97	0.78	0.92	0.61
Proline	3.21	4.15	5.26	3.75	4.12	3.31	4.01	5.23	4.25	5.12	4.13	5.62	5.92	5.74	5.74
Glutamic	3.52	4.23	4.89	3.78	3.92	3.62	3.90	4.35	4.32	4.34	4.31	4.73	4.95	4.71	4.92
Alanine	1.23	1.12	0.95	1.45	1.68	1.42	1.62	1.12	1.22	1.07	1.23	1.32	1.41	1.32	1.40
Histidine	0.94	0.82	0.57	0.84	0.94	0.83	0.92	0.67	0.97	0.96	0.94	0.88	0.51	0.88	0.43
Arginine	0.95	1.99	2.42	0.99	2.45	1.34	2.42	1.87	2.35	1.68	2.30	2.46	2.54	2.41	2.50
Cystine	0.56	0.28	0.24	0.34	0.42	0.31	0.38	0.29	0.32	0.27	0.31	0.22	0.19	0.21	0.18
Methionine	0.62	0.60	0.32	0.65	0.74	0.64	0.69	0.54	0.43	0.51	0.41	0.22	0.21	0.22	0.20
Valine	1.69	1.53	0.90	1.76	1.89	1.74	1.92	1.52	1.67	1.32	1.61	1.21	0.89	1.11	0.81
Tyrosine	0.77	0.56	0.13	0.88	0.94	0.80	0.88	0.62	0.75	0.60	0.72	0.26	0.19	0.31	0.19
Phynyl alanine	1.79	1.51	0.82	1.38	1.48	1.51	1.62	1.41	1.57	1.32	1.39	0.99	0.89	0.94	0.89
Lysine	1.21	1.11	0.81	1.74	1.95	1.63	1.90	1.10	1.23	1.00	1.21	0.95	0.65	0.91	0.62

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