

# Effect of salicylic acid on soluble sugars, proline and protein patterns of shara (*Plectranthus tenuiflorus*) plants grown under water stress conditions

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## Abstract

Experiments were conducted to study the effect of 0.5 mM salicylic acid (SA) on growth, osmoregulating solutes (proline and soluble sugars), and protein patterns of Shara (*Plectranthus tenuiflorus*) plants grown under different levels of irrigation water (100, 80, 60 and 40% of the field capacity, FC). Water stress, particularly at 40% FC, decreased fresh and dry weights in the absence of SA. Values of, sugars and proteins, were decreased by severe water stress while SA treatments improved these values. Proline content increased under water stress. The electrophoresis studies showed that some new protein bands were observed propably to increase plant tolerance against water stress effect.

**Keywords:** Drought stress, shara plant, salicylic acid (SA), protein patterns.

## INTRODUCTION

Shara (*Plectranthus tenuiflorus*) plant is one of the medicinal herbs that have a promising economical future in the Kingdom of Saudi Arabia and is cultivated as a source of essential oils, and as edible tubers, or food flavorings (Khorshid *et al.*, 2011). The components of the essential oil of *P. tenuiflorus* have an antimicrobial effect and are used in folk medicine, they are employed for headaches, sores, burns, dermatitis, acute edematous, stomachache, against nausea, scorpion stings and as purgative (Rahman *et al.*, 2004).

In an early study, Mwangi *et al.* (1993) found that the essential oil of *P. tenuiflorus* from Kenya contained  $\alpha$ -terpinene (10.2%), p-cymene (10.9%) and carvacrol (14.3%) as the major components, while Al-Yahya (2004) found that the essential oil of *P. tenuiflorus* grown in Saudi Arabia contained  $\Delta^3$ -Carene (58%) as a major component. But, Khorshid *et al.* (2011) has reported that the principle component of the oil produced from plant harvested in Saudi Arabia was found to be Thymol (85.3%). Therefore, the subject of oil components of Shara plants still unclear and needs further studies.

Drought conditions are dominant in Saudi Arabia as in other arid and semi-arid countries. Severe water stress reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Farooq *et al.*, 2009). The most obvious effect of water stress is growth reduction (Amin *et al.*, 2009). Thus, a common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al.*, 2009). Drought stress causes closing stoma and reducing leaf area and photosynthetic pigments (Amin *et al.*, 2009).

Several plant species accumulate organic solutes such as sugars and proline in response to water and/or osmotic stress. Proline accumulation might respond to various stresses such as drought and salinity, hence it has been used for one of the indicating parameter of stress tolerance selection in plants (Chutipaijit *et al.*, 2008). Salicylic acid (SA) is an endogenous regulator of phenolic nature, which is involved in the regulation of various physiological processes in plants (Shakirova *et al.*, 2003) and in alleviating the deleterious effect of drought stress (Bideshki and Arvin, 2010). It improves plant growth (Khodary, 2004), enzyme activities and ion uptake and transport (Khan *et al.*, 2010). Results of early

studies suggested that exogenously applied SA could induce drought resistance in wheat, bean and tomato (Waseem *et al.*, 2006). The important protective action of SA probably reflects its ability to induce the expression of genes coding for proteins (Shakirova, 2003). Data exist about SA induced synthesis of heat shock proteins in tobacco plants, accumulation of wheat lectins and fast activation of the 48 kDa protein kinase in suspension cell cultures of tobacco under stress demonstrating the involvement of SA in the induction of different antistress programs (Shakirova, 2003).

The aim of the present work was to determine the effect of salicylic acid (SA) on growth, soluble sugars, proline and protein patterns of *P. tenuiflorus* plants grown under water stress conditions.

## MATERIALS AND METHODS

### Experiments

The present study was carried out at the King Abdulaziz University Experimental Station, Jeddah; Saudi Arabia during summer seasons (June - September) of 2010 and 2011 which is usually characterized by sunny, hot, dry days and warm nights. The average daily maximum temperature was 37.6°C with little variation. The daily minimum temperature during the experiment ranged from 15.6 to 28.5°C. No rain falls during the experimental periods. The study aimed to determine the effect of SA on growth, photosynthetic pigments and essential oil components of Shara (*P. tenuiflorus*) plants grown under water stress conditions.

Cuts of Shara (*Plectranthus tenuiflorus*) stems were grown in plastic pots (25 cm diameter) filled with soil mixed of sand and peatmoss (3:1). The soil pH in the pots was adjusted to 6.8 -7.2 for normal plant growth.

Plants were grown outdoors under natural conditions in a shade-free location. Plants were watered to field capacity for about one month until roots were established and shoot system was in a reasonable size. Soil surfaces in the pots were covered with white polyethylene film to minimize evaporation.

A completely randomized design with 10 replicates for each treatment was used in this experiment. Plants of uniform height (one plant per pot) were selected and located in lines with a spacing of 2 m between lines and 1 m between pots to avoid mutual shading. The ground surface between and surrounding the pots consisted of bare soil.

Three weeks after transplanting, all seedlings were fertilized 3 times, within 10 day intervals, with the complete water-soluble fertilizer "Sangral" compound fertilizer (20N-20P-20K, plus micronutrients) at the rate of 2g/kg soil.

For the first 4 weeks, pots were weighed every other day, and all plants were watered to field capacity (FC),

supplying an amount of water equal to transpiration losses; to ensure the establishment of seedlings and to allow adaptation to surround conditions before water stress was imposed. By the end of this period, watering was discontinued and pots were divided into two groups one of them was sprayed with 0.5 mM salicylic acid (SA) as recommended by Németh *et al.* (2008) and the other group was left untreated. Each group was, in turn, divided into 4 sub-groups which received an amount of water equal to 100% (control), 80%, 60% or 40% of the field capacity and allowed to grow for 4 more weeks, during which plants were sprayed 3 times with SA. The field capacity was determined gravimetrically and found to be 16%; each water treatment consisted of 10 replicates to make up 80 pots for the whole experiment.

### Measurements

One week after the last SA treatment the following parameters were measured:

#### Total fresh and dry weights

Dry weights of plant shoots and roots were determined after drying the fresh plant organs in electrical oven at 70°C until constant weight. Weights were recorded at three growth stages, seedling stage (stage I), mature stage (stage II) and harvest stage (stage III). The decision to harvest any particular treatment was based on the beginning of death symptoms.

#### Soluble sugars

Soluble sugars were determined spectrophotometrically using the phenol-sulfuric acid method described by Dubois *et al.* (1956). Standard curves with glucose were prepared and the contribution of soluble sugars was calculated based on the dry weight bases.

#### Free proline

Concentrations of free proline were determined according to Bates *et al.* (1973). Leaf samples (approximately 0.2 g) were homogenized in 10 mL of 3% (v/v) aqueous sulfosalicylic acid. The homogenate was filtered through Whatman 41 filter paper. The filtrate, was acidified with glacial acetic acid and ninhydrin (1 mL each) and was heated in water bath at 100 °C for 1 h. The mixture was extracted with 5 mL toluene and the upper (toluene) phase decanted into a glass cuvette and the absorbance was measured at 520 nm (red light). Proline concentrations were calculated using proline standards (0–50 µg/ml) in identical manner.

**Table 1.** Effect of drought and salicylic acid (SA) on total fresh weight (g) of *Plectranthus tenuiflorus* plants at different stages of growth

| Field capacity | Stage I  | Stage II | Stage III |
|----------------|----------|----------|-----------|
| 100%           | 124.2 c  | 251.2 b  | 348.4 c   |
| 80%            | 126.3 c  | 227.8 c  | 321.2 d   |
| 60%            | 111.8 d  | 165.6 d  | 234.2 f   |
| 40%            | 085.2 e  | 140.6 e  | 176.8 g   |
| 100% + SA      | 140.4 b  | 268.8 b  | 443.7 b   |
| 80% + SA       | 169.2 a  | 301.1 a  | 509.2 a   |
| 60% + SA       | 132.7 bc | 208.5 c  | 345.9 c   |
| 40% + SA       | 095.1 e  | 184.2 d  | 288.3 e   |

Means in the same column that have the same letter are not significantly different at  $P < 0.05$

**Table 2.** Effect of drought and salicylic acid (SA) on total dry weight (g) of *Plectranthus tenuiflorus* plants at different stages of growth

| Field capacity | Stage I | Stage II | Stage III |
|----------------|---------|----------|-----------|
| 100%           | 6.47 c  | 17.80 bc | 36.00 c   |
| 80%            | 7.10 bc | 16.80 bc | 32.40 c   |
| 60%            | 5.40 d  | 12.40 de | 23.70 d   |
| 40%            | 4.40 d  | 10.00 e  | 16.20 e   |
| 100% + SA      | 7.60 b  | 19.30 ab | 43.00 b   |
| 80% + SA       | 9.20 a  | 22.00 a  | 47.80 a   |
| 60% + SA       | 7.20 bc | 15.00 cd | 34.70 c   |
| 40% + SA       | 5.27 d  | 13.30 d  | 26.60 d   |

Means in the same column that have the same letter are not significantly different at  $P < 0.05$

## Total protein

Total protein was analyzed by measuring nitrogen concentrations using Kjeldahl procedure then the percentages of total nitrogen were converted to protein values by multiplying nitrogen content by the factor 6.25 according to Sriperm et al. (2011).

## Protein patterns

Shoot samples were homogenized and extracted in 50 mM sodium phosphate buffer (pH 7.5). Protein samples were prepared by mixing the extract with 2X SDS-PAGE treatment buffer and boiled for 4 min. The denatured protein samples were analyzed by vertical one dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE); Protein bands in the gel were visualized by a Coomassie Brilliant Blue R-250 (CB) with Bismarck Brown R (BBR) according to Choi et al. (1999).

## Statistical analysis

The experiment was arranged in a completely randomized design and was analyzed by analysis of variance. All data were statistically analyzed and ANOVA was tested according to Snedecor and Cochran (1980) with the aid of SPSS (1990) computer program for statistics. Differences among treatments were tested with LSD at 5% level of significance.

## RESULTS AND DISCUSSION

### Fresh and dry weights

Data recorded in Table 1 and 2 indicated clearly that total fresh and dry weights decreased drastically under water stress condition compared with unstressed condition. In the absence of SA, the 40% FC reduced the total fresh weights by about 31, 44 and 47% at stage I, II and III, respectively, as compared with control (100% FC). The

**Table 3.** Effect of drought and salicylic acid (SA) on soluble sugar ( $\text{mg}^{-1}\text{g dwt}$ ) of *Plectranthus tenuiflorus* shoots

| Field capacity | Stage I | Stage II | Stage III |
|----------------|---------|----------|-----------|
| 100%           | 2.74 ab | 6.71 a   | 5.87 b    |
| 80%            | 2.53 bc | 5.81 b   | 4.23 c    |
| 60%            | 1.94 c  | 3.67 c   | 3.11 d    |
| 40%            | 1.12 d  | 2.42 e   | 2.23 e    |
| 100% + SA      | 2.85 ab | 6.88 a   | 6.19 b    |
| 80% + SA       | 3.45 a  | 7.11 a   | 6.87 a    |
| 60% + SA       | 2.43 bc | 4.66 c   | 4.12 c    |
| 40% + SA       | 1.82 cd | 3.75 d   | 3.21 d    |

Means in the same column that have the same letter are not significantly different at  $P < 0.05$

corresponding reductions in total dry weights were about 20%, 35% and 40%, respectively. While, SA treatments improved total plant weights. In this regard the decrease in total fresh weights at 40%FC+SA were 23, 27 and 17%, respectively. The decrease in total dry weights at the same treatment were 18, 25 and 26%, respectively.

The present study showed that total fresh and dry weights of Shara plants substantially decreased under severe water stress. Similarly, Bideshki and Arvin (2010) found that drought reduced total fresh weight of garlic by about 40%, relative to control well watered plants. The reduction in plant growth under water stress may be attributed to the negative effect of drought on cell turgor pressure and cell expansion rate, a decline in metabolic activity of plant cells, which cause inhibition of their growth (Amin *et al.*, 2009) and/or reduction in photosynthesis and disturbance in the accumulation of nutrients (Steduto *et al.*, 2000).

The positive effect of SA on plant growth under water stress, observed in the present study, was similar to that of Khodary (2004) who reported that SA increased the fresh and dry weight of shoot and roots of stressed maize plants. Increased fresh and dry weights of water stressed plants in response to SA was attributed to the induction of antioxidant responses that protect the plant from damage (Senaratna *et al.*, 2000) and was also ascribed to increased photosynthesis and nutrient content (Khan *et al.*, 2010).

### Soluble sugars

The adverse effect of water stress on soluble sugars increased as drought became more severe (Table 3). In this regard, the levels of soluble sugars at 60% FC were about 70%, 55% and 50% of the control (100% FC) at stages I, II and III, respectively. While the corresponding values at 40% FC were about 40%, 36% and 38% of the

control, respectively.

SA, on the other side, enhanced the soluble sugar accumulation in both water-stressed and non-stressed plants. At 100% FC sugar concentration increased 4-5% when plants were treated with SA at the different growth stages. At (80% FC+SA) treatment sugars increased by about 35%, 18% and 60% at stage I, II and III, respectively. The corresponding increase at 60% FC was 25, 20 and 30%, respectively, when plants treated with SA. The increase at 40% FC was more observed and reached 60, 50 and 40%, respectively.

This study cleared that drought stress was associated with reduction in sugar concentration and when levels of dryness increased, sugar content decreased more. The reduction of sugar content under water deficit may be attributed to the negative effect of drought stress on photosynthetic pigments and photosynthesis (Yazdanpanah *et al.*, 2011) who found that net photosynthesis, transpiration rate and stomatal conductance were significantly affected by stress due to changes in chlorophyll content and chloroplast structure. In a previous study by Jalal *et al.* (2012) they found that 60% and 40% FC decreased chl a, chl b, carotenoids and caused stomatal closure in *P. tenuiflorus* plants. Stomatal closure, in turn, restricts  $\text{CO}_2$  entry into leaves thereby decreasing  $\text{CO}_2$  assimilation and rate of transpiration (Chaves, 2002).

In agreement with Miguel *et al.* (2006) findings, the present study showed that SA treatment improved plant tolerance against water stress and sugars approached near its normal condition. Increasing amount of sugars and thus the osmosis gradient in plant tissues treated with SA would lead to the resistance against losing water, protect chloroplasts and accelerate plant growth under stress conditions (Amin *et al.*, 2009). The exogenous application of SA counteracted the drought deleterious effects on sugars in maize (Khodary, 2004) and okra (Amin *et al.*, 2009).

**Table 4.** Effect of drought and salicylic acid (SA) on proline content (mg<sup>-1</sup>g fwt) of *Plectranthus tenuiflorus* leaves

| Field capacity | Stage I | Stage II | Stage III |
|----------------|---------|----------|-----------|
| 100%           | 2.42 e  | 3.14 d   | 3.54 e    |
| 80%            | 2.55 e  | 3.25 d   | 4.12 d    |
| 60%            | 3.12 cd | 6.23 b   | 6.85 b    |
| 40%            | 2.97 d  | 4.31 c   | 5.25 c    |
| 100% + SA      | 2.56 bc | 3.88 cd  | 4.22 d    |
| 80% + SA       | 3.11 cd | 4.27 c   | 5.65 c    |
| 60% + SA       | 4.21 ab | 8.43 a   | 10.12 a   |
| 40% + SA       | 4.86 a  | 8.50 a   | 11.54 a   |

Means in the same column that have the same letter are not significantly different at  $P < 0.05$

**Table 5.** Effect of drought and salicylic acid (SA) on protein content (mg<sup>-1</sup>g fwt) of *Plectranthus tenuiflorus* leaves

| Field capacity | Stage I | Stage II | Stage III |
|----------------|---------|----------|-----------|
| 100%           | 2.6 ab  | 4.0 ab   | 3.9 c     |
| 80%            | 2.8 ab  | 3.8 ab   | 4.1 bc    |
| 60%            | 2.1 b   | 2.8 bc   | 2.4 de    |
| 40%            | 1.5 b   | 2.2 c    | 1.9 e     |
| 100% + SA      | 3.9 a   | 4.7 a    | 4.6 b     |
| 80% + SA       | 4.1 a   | 4.9 a    | 5.2 a     |
| 60% + SA       | 3.8 a   | 4.0 ab   | 3.7 c     |
| 40% + SA       | 2.6 ab  | 3.1 bc   | 2.6 d     |

Means in the same column that have the same letter are not significantly different at  $P < 0.05$

### Proline content

Data in Table 4 shows that with the increase of water stress up to 80% and 60% FC, proline increased significantly as compared with control plants (100% FC), but at severe stress (40% FC) its levels decreased. This was observed all over the three growth stages of the plant. Data also illustrated that when plants were kept under drought stress, the amount of proline would increase. This increase will become so great with an increase in the period of drought stress. In this regard, proline level increased with plant age to reach its maximum value at stage III.

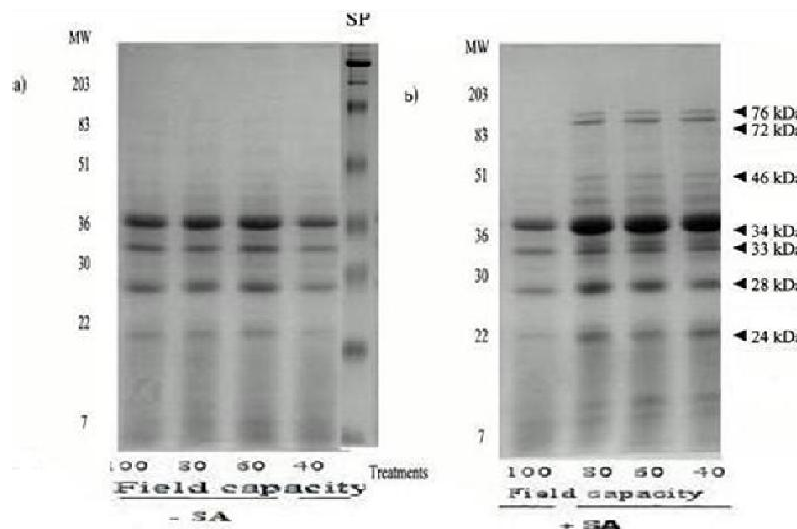
SA treatment increased proline concentration whether plants were under stress or not. However, the most effect of SA on proline content was more pronounced in stressed than that in non-stressed plants. The (100% FC+SA) treatment increased proline concentration by about 6, 25 and 20%, at stage I, II and III, respectively, as compared with (100% FC) treatment at same stages.

The present study showed that mild water stress increased proline while severe water stress affected

negatively the level of proline in stressed Shara plants. In this regard, Yazdanpanah *et al.* (2011) reported that the amount of proline increased under mild drought stress because proline is a key in osmosis regulation. Moreover, our study showed that SA stimulates proline accumulation in water stressed plants. Increasing the amount of proline and sugars in the plants would lead to the resistance against losing water, protect turgor, reduce the membrane damage and accelerate the growth of plants in stress conditions (Amin *et al.*, 2009). The higher accumulation of proline under stress conditions was attributed to enhanced activities of proline biosynthesis enzymes, ornithine aminotransferase and pyrroline-5-carboxylate reductase, as well as due to inhibition of proline degradation enzymes, proline oxidase and proline dehydrogenase (Kishor *et al.*, 2005).

### Protein content

It is obvious from the data in Table 5 that drought stress decreased protein content considerably, but SA treatment



**Figure 1.** Analysis of protein patterns by one-dimensional SDS-PAGE

lessened this negative effect and improved protein content. The reduction in protein content was more observed at severe water stress compared to mild stress. In this regard, the least values of protein were observed at 40% FC, at which protein content decreased by about 42, 45 and 50% at growth stage I, II and III, respectively, as compared with 100% FC.

Clearly, SA increased protein concentration either in stressed or non-stressed plants Table 5. The increase in protein content at (100% FC+SA) treatment was about 50, 18 and 15% at stage I, II and III, respectively. The higher effect of SA on protein was more observed at high water stress than at low or mild water stress. The percent increase in protein content at (40% FC+SA) treatment was 66%, 40% and 36% at stage I, II and III, respectively, as compared with the values recorded at 40% FC without SA.

Many studies showed that protein concentrations decreased in many plant species under drought stress (Hussein *et al.*, 2007) due to decreasing protein biosynthesis (Amin *et al.*, 2009) and enhancing degradation (Black and Prithard, 2002). A common effect of drought stress is to cause oxidative damage (Smirnoff 1998). The reactive oxygen species (ROS), produced at water stress conditions, enhance decomposition of proteins (Yazdanpanah *et al.*, 2011). The accumulation of ROS during drought stress, along with increasing  $H_2O_2$ , often enhances protein oxidation in plant species (Jiang and Nhunag, 2001). While, other investigators reported that reducing protein content was a result of the negative effect of drought on the nitrate reductase activity and nitrogen metabolism (Ahmad *et al.*, 2003). The present study showed that SA treatment could relieve the negative effect of stress on protein and increased its values. It was reported that SA alleviate the harmful effect of water

stress through increasing the antioxidant compounds and enhancing the activity of antioxidant enzymes as well as stimulating new proteins synthesis (Avancini *et al.*, 2003). Moreover, Ahmad *et al.* (2003) showed that SA increased nitrate reductase activity and thus stimulate protein formation.

### Protein patterns

Changes of protein patterns have been analyzed in leaves of shara plants (Figure 1), in order to follow any possible alterations in gene expression in plants subjected to drought stress at different field capacity levels in the absence or presence of SA comparing with non-stressed control at 100% FC.

It is clear that water stress and SA treated plants induced variations in the appearance of new protein bands and in disappearance of others with different high molecular weights, whereas no changes in protein patterns with low molecular weights were observed. The SA treatment indicated that (1) in non-stressed plants; application of SA did not change the pattern of protein bands, while (2) In drought-stressed plants; SA treatment induced the appearance of two new polypeptides (73 and 76 kDa). It appears clearly that SA treatment at 80%, 60% and 40% FC enhanced the formation of a 73 and 76-kDa proteins. In contrast, synthesis of these proteins was negatively affected by sufficient watering (100% FC) and by water stress without SA treatments as shown in panel (a).

The new bands of high molecular weight proteins in drought stressed plants treated with SA might be due to *de novo* synthesis of these proteins (Gopala Roa *et al.*, 1987). These new proteins may have a specific function

to protect shara plants from further dehydration damage and considered as a defense mechanism to drought stress. Drought induced polypeptides have been observed in many studies and are assumed to play a role in water stress tolerance (Jiang and Huang, 2002).

Figure1, Analysis of protein patterns by one-dimensional SDS-PAGE extracted from leaves of Shara (*Plectranthus tenuiflorus* L.) plants grown under different watering regimes (100, 80, 60 and 40% FC) and untreated (-SA) or treated (+SA) with salicylic acid (SA). The effect of salicylic acid on the approximately 73 and 76 MW protein in the leaves of shara plants (lanes 2b to 4b) is shown. Lanes (SP) on the left contained marker proteins whose molecular masses (kilodaltons) are shown on the right side of the panels. Salicylic acid was sprayed at 0.5 mM (b) on shara plants grown under different watering regimes (100%, 80%, 60% and 40% FC). The lane on the left side indicates the molecular weights (MW) of standard proteins (SP). The lane on the right is used to specify MW of proteins indicated by arrows.

Disappearance of certain polypeptides in drought-stressed plants in the absence of SA may be related to increase the hydrolyzing enzyme RNAase activity (Kong-ngern et al, 2005). The effect of SA was clear, suggesting an interaction between the protein synthesis and SA.

It is also clear that the severe drought stress treatment (40% FC) did not affect the accumulation of the protein subunits between 25 and 38 KDa (panel a), however, these subunits, particularly that found at 38 KDa, were so condensed when water stressed plants were treated with SA (Panel b). These data suggest that accumulation of the low MW subunits proteins (between 37- 36 KDa) was insensitive to drought stress, therefore they appear either under water unstressed or water stressed samples. Nevertheless, drought stress might have accounted for the delayed onset of high MW protein subunits, relative to the onset of the low MW subunits, under water stressed in the absence of SA treatments, therefore the 73 and 76 KDa subunits were formed in SA-treated samples even though they were under severe water stress condition. These results were consistent with Samarah *et al.* (2006), who reported that, soybean seeds produced under drought stress had a variation in  $\beta$ -subunit of the  $\beta$ -conglycinin, probably because of degradation of proteins in the shriveled seeds produced under drought stress.

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