
Effect of salt and acid stress on *Triticum aestivum* inoculated with *Glomus fasciculatum*

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Arbuscular mycorrhizal (AM) symbiosis increased resistance to various abiotic stresses, such as drought, acid and salt stresses. The effect of salt and acid stress on *Triticum aestivum* L. varieties was studied with and without mycorrhizal inoculation. Four *T. aestivum* L. varieties were subjected to salt stress by supplementing 1N NaCl and acid stress by 1N HCl under control and inoculated conditions. Plant growth was reduced in control plants under salt and acid stress. AM inoculation helped the plants to withstand acid and salt stress. An inoculated plant showed better growth under salt and acid stress than control plants. Acid and salt treatments were found to be inhibitory for growth and development of *Triticum aestivum* L. varieties. Acid stress was found to be more inhibited than salt stress. Less growth was noticed in plants subjected to acid stress.

Key words: Arbuscular mycorrhizal fungi, *Triticum aestivum* L. varieties, HCL and NaCl

Introduction

AM fungi are known to facilitate water and mineral uptake by the host plants under normal and stress conditions (Gupta and Krishnamurthy, 1996; Morte *et al.*, 2000). Several studies have demonstrated that AM symbiosis can improve resistance to various abiotic stresses (Azcon and El Atrash, 2000; Porcel *et al.*, 2003). AM fungi help to overcome resistance to various salt stresses by increasing the water and nutrient uptake from the soil. Salt and acid tolerance of plants is a complex phenomenon that involves physiological, biochemical and molecular changes. Reduction in growth and yield are undoubtedly the most important physiological response of plants to the excess salt in the media. Salt resistance was improved by AM colonization in Maize (Feng *et al.*, 2002), mung bean and clover with the AM fungal effect correlated with improved osmoregulation or proline accumulation. AM colonization also improved NaCl resistance in Tomato, with extent of improvement related to salt sensitivity of the cultivar (Al-Karaki, *et al.*, 2001). There is considerable

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evidence to suggest that AM fungi are able to increase the host plant's tolerance to water stress (Davies *et al.*, 1992; Smith and Read, 1997; Auge, 2001), including that caused by high salinity (Feng *et al.*, 2002). Soil salinity affects crop plants in three major ways, osmotic stress results in decreasing water availability, ionic stress and changes in the cellular ionic balance. Physiologically many processes are affected but notably these are reduced cell growth, decreased leaf area, biomass, and yield. Wheat has a moderate tolerance to salinity.

AM Fungus selected for the study was *Glomus fasciculatum*. It was an efficient AM fungus with which the *Triticum aestivum* L. var. shows better growth and development. Four *Triticum aestivum* L. var. commonly cultivated in North Karnatak DWR162, DWR 195, DWR 225 and NI 5439 were selected for the experiments. The germ plasm of these varieties was collected from University of Agricultural Sciences, Dharwad. The objective of this study was to elucidate how salt and acid stress influences the growth of AM fungus and the host plants. An attempt has been made to study the stress tolerance of indigenous AM fungus and its impact on the growth and development of *Triticum aestivum* L.

Materials and methods

Source of AM inoculum

The AM fungus *Glomus fasciculatum* was isolated according to Gerdmann and Nicolson (1963) method. This AM fungus was mass multiplied by using *Sorgum vulgare* L. grown on sterile soil. Finally three month old multiplied AM inoculum was used for the experiment.

Experimental design

Experiments were conducted in earthen pots measuring 20 cm diameter. The sterilized soil and sand was mixed in 1:1 ratio and filled in the experimental pots. Seeds of four *Triticum aestivum* L. var. DWR 162, DWR 195, DWR 225 and NI 5439 were selected for the experiments. The germ plasm of these varieties was collected from University of Agricultural Sciences Dharwad. Seeds were sterilized with 2% sodium hypochloride solution. To remove the traces of sodium hypochloride seeds were washed with distilled water four times. About ten seeds were placed in each pot. Control pots were not added with AM fungal inoculum. Plants were inoculated with AM fungus, before sowing the seeds, inoculum of *Glomus fasciculatum* was placed 2 cm

below the soil. Experiments were conducted in six groups, each group was maintained in triplicates as follows:-

- Group 1.** Control plants were grown in pots containing sterilized soil and sand mixture without inoculum. Plants were regularly watered on alternate days.
- Group 2.** Plants were grown in pots containing sterilized soil and sand mixture with AM Fungal inoculum. Plants were regularly watered on alternate days.
- Group 3.** Plants were grown in pots containing sterilized soil and sand mixture without inoculum. Plants were regularly watered on alternate days and are treated with 25 ml of NaCl (3%) solution per pot once in a week.
- Group 4.** Plants were grown in pots containing sterilized soil and sand mixture with AM inoculum. Plants were regularly watered on alternate days and are treated with 25ml of NaCl (3%) solution per pot once in a week.
- Group 5.** Plants were grown in pots containing sterilized soil and sand mixture without AM inoculum. Plants were regularly watered on alternate days and are treated with 25ml of 0.5% HCl per pot once in a week.
- Group 6.** Plants were grown in pots containing sterilized soil and sand mixture with AM inoculum. Plants were regularly watered on alternate days and are treated with 25ml of 0.5% HCl per pot once in a week.

Pots belonging to four *Triticum aestivum* L. Var. DWR 162, DWR 195, DWR 225 and NI 5439 were maintained in triplicates with above mentioned treatments. Altogether 72 pots were maintained and watered on alternate days to maintain sufficient moisture. Acid and salt stress were induced by treating the plants with NaCl and HCl respectively after 15 days from the date of sowing.

Growth parameters

Growth parameters of *Triticum aestivum* L. var. like plant height, girth of stem, shoot biomass, root biomass, leaf number and leaf length were measured in 60 days old plants.

Determination of root colonization

AM fungal colonization in the roots of *Triticum aestivum* L. var. grown under different treatments were determined by Philips and Hayman method. Roots are washed with 10% KOH solution and stained with 0.05% (V/V) tryphan blue in lactophenol. 30 randomly chosen root fragments of 1cm length were mounted on slide and examined microscopically. Per cent of mycorrhizal colonization was determined using following formula.

$$\text{Root colonization (\%)} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

Determination of spore count

Total spore count was determined by wet sieving and decanting method.

Statistical analyses

The data were statistically analyzed using Analysis of Variance (ANOVA) and the means were separated by Duncan's Multiple Range Test (DMRT) using SPSS 7.5.

Results

Mycorrhizal colonization

The non-inoculated plants grown with and without salt and acid stress showed mycorrhizal colonization. Varied degree of colonization was found in inoculated plants grown with and without salt and acid stress. A very high per cent of root colonization was observed in inoculated plants which was not subjected to any stress. The four tested varieties of *Triticum aestivum* L. showed 80-85% of root colonization that observed in inoculated plants grown without salt and acid stress. Inoculated plants grown under salt stress showed lesser colonization, which was about 60-70%. Inoculated plants treated with acid exhibited very less per cent of root colonization than the plants grown without stress and with salt stress. These experiments it was evident that salt stress resulted in the decrease in mycorrhizal colonization to moderate extent. But the acid stress was found to be lethal for mycorrhizal colonization, resulted in significantly decreased in mycorrhizal colonization.

Number of spores and vesicles

Number of spores presenting in the rhizosphere was found proportional to the extent of mycorrhizal colonization. Spore number was counted per 100 gm soil. Maximum spore number was observed in stress free plants, which were not subjected to acid or salt stress. The spore number was found approximately 150 per 100 gm soil. The rhizosphere of plants treated with NaCl were subjected to salt stress that showed comparatively lesser spore number, which was found approximately 80 per 100 gm soil. The rhizosphere of plants subjected to acid stress showed least spore number, which was about 15-20 spores per 100gm soil. The vesicles showed a maximum number in inoculated plants without stress. Plants treated with NaCl showed less number of vesicles than stress free plants. Acid treatment resulted in least vesicle formation. The number of vesicles produced in acid treated plants was very least. Salt and acid treatments were not only reduced the mycorrhizal colonization, spore number but also the number of vesicles.

Growth parameters of *Triticum aestivum* L.

The effect of *Glomus fasciculatum* on *Triticum aestivum* L. was measured with the consideration of morphological parameters like plant height, stem diameter, root biomass, shoot biomass leaf number, and leaf length in 60 days old plants. Inoculated plants demonstrated better performance and growth parameters than uninoculated plants. Salt and acid stress resulted in reduced growth than untreated plants. Plants colonized by *G. fasciculatum* increased in parameters than uninoculated plants. Maximum plant height was observed in inoculated and stress free plants belong to DWR 225 (6.233 ± 0.120) and DWR 162 (6.40 ± 0.05). A very least plant growth was observed in acid treated uninoculated plants. Among acid treated uninoculated plants DWR 195 and DWR 162 were shown the least plant height (Table 2 and 3). Plants subjected to salt stress were shown a better plant height than plants subjected to acid stress. Plants treated with NaCl inoculated with *G. fasciculatum* were shown to be more plant height than uninoculated plants. The results revealed that AM fungal inoculation minimized the effect of salt and acid stress in all the four *T. aestivum* L. var. (Fig. 1). Stem diameter, leaf number, leaf length, shoot and root biomass were found to be higher in inoculated stress free plants. The uninoculated stress free plants showed lesser parameters than inoculated plants. DWR 225 showed maximum shoot biomass (Table 4) and DWR 162 showed maximum root biomass under inoculated conditions without stress (Table 2). Lesser root and shoot biomass was observed in uninoculated plants without stress. Plants subjected to salt and acid stress exhibit lesser root and shoot

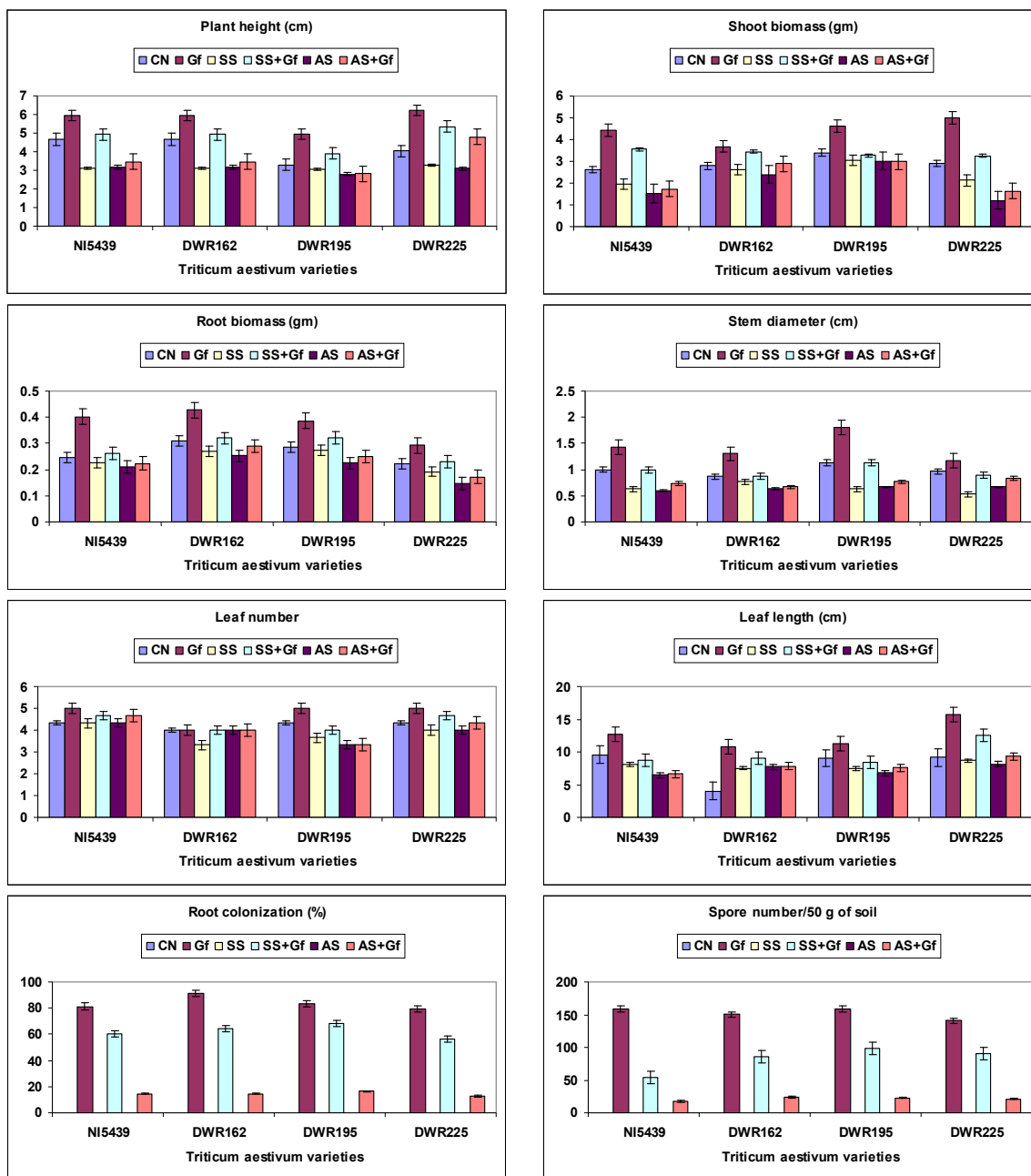
biomass than stress free plants both under inoculated and uninoculated plants (Fig. 1). Plants inoculated with AM fungus (*Glomus fasciculatum*) showed comparatively higher root and shoot biomass than uninoculated plants under salt and acid stress conditions (Fig. 1). Acid stress was found to be more deleterious than salt stress plants showed lesser growth parameters in presence of acid stress. AM inoculation did not improve the growth of plants treated with acid, there was not much difference in the growth parameters of inoculated and uninoculated plants treated with acid. Over all plant growth showed the least promoted in inoculated plants treated with acid. AM fungal inoculation promoted overall growth of plants to the extent of 30 to 50% in salt treated plants. Leaf number did not exhibit much different in plants subjected to various treatments. Leaf length was found to minimum in uninoculated acid treated plants and maximum in AM fungal inoculated stress free plants (Fig 1).

Table 1. The effect of salt and acid stress on *Triticum aestivum* L. var. NI 5439 inoculated with *Glomus fasciculatum*.

Treatments	Plant height	Stem Diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	4.66±0.088b	1.00±0.152b	2.62±0.095c	0.245±0.007b	4.33±0.333ab	9.6±0.296b	0.0±0.00d	0.0±0.00d
Inoculated untreated	5.93±0.208a	1.433±0.120a	4.43±0.133a	0.402±0.016a	5.0±0.000a	12.63±0.463a	81.20±10.30a	159±20.56a
Salt stress without inoculation	3.13±0.218c	0.633±0.066c	1.96±0.175d	0.226±0.004b	4.33±0.333ab	8.06±0.284b	0.0±0.00d	0.0±0.00d
Salt stress with inoculation	4.93±0.240b	1.00±0.057b	3.56±0.159b	0.262±0.029b	4.66±0.333ab	8.766±0.233b	60.40±9.53b	54.00±4.00b
Acid stress without inoculation	3.166±0.284c	0.600±0.054c	1.516±0.090e	0.211±0.019c	4.33±0.333ab	6.46±0.567d	0.0±0.00d	0.0±0.00d
Acid stress with inoculation	3.466±0.338c	0.733±0.30bc	1.723±0.128de	0.224±0.004c	4.66±0.333ab	6.66±0.504c	14.66±1.32c	17.33±1.85c

Table 2. The effect of salt and acid stress on *Triticum aestivum* L. var. DWR 162 inoculated with *Glomus fasciculatum*.

Treatments	Plant height	Stem Diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	4.66±0.088b	0.866±0.033b	2.80±0.30bc	0.311±0.013b	4.0±0.00a	4.0±0.00a	0.0±0.00d	0.0±0.00d
Inoculated untreated	5.93±0.208a	1.300±0.115a	3.69±0.349a	0.427±0.043a	4.0±0.00a	10.83±0.28a	91.03±3.80a	150.33±9.7a
Salt stress without inoculation	3.13±0.218c	0.766±0.033b	2.63±0.318bc	0.270±0.022c	3.33±0.33b	7.53±0.145c	0.0±0.00d	0.0±0.00d
Salt stress with inoculation	4.93±0.240b	0.866±0.033b	3.43±0.120ab	0.320±0.002b	4.0±0.00a	9.03±0.233b	64.0±5.56b	86.0±2.08b
Acid stress without inoculation	3.166±0.284c	0.633±0.033c	2.40±0.300c	0.253±0.012c	4.0±0.00a	7.73±0.338c	0.0±0.00d	0.0±0.00d
Acid stress with inoculation	3.466±0.338c	0.667±0.033c	2.89±0.261b	0.289±0.019c	4.0±0.00a	7.83±0.338c	14.66±1.26c	23.66±4.25c



CN=Control; Gf=*Glomus fasciculatum*; SS=Salt stress; AS=Acid stress;

Fig 1. The effect of salt and acid stress on *Triticum aestivum* L. var. under control and inoculated conditions.



Fig. 1. The effect of *Glomus fasciculatum* on NI 5439 var. under control and inoculated conditions without stress.



Fig. 2. The effect of *Glomus fasciculatum* on DWR 225 var. under control and inoculated conditions with salt stress.



Fig. 3. The effect of *Glomus fasciculatum* on DWR 195 var. under control and inoculated conditions with acid stress.

Table 3. The effect of salt and acid stress on *Triticum aestivum* L. var. DWR 195 inoculated with *Glomus fasciculatum*.

Treatments	Plant height	Stem Diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	3.30±0.115bc	1.13±0.066b	3.386±0.095b	0.287±0.027bc	4.33±0.333ab	9.06±0.338b	0.0±0.00d	0.0±0.00d
Inoculated untreated	4.93±0.650a	1.80±0.057a	4.62±0.298a	0.386±0.019a	5.00±0.57a	11.23±0.218a	83.41±5.46a	158.66±4.19a
Salt stress without inoculation	3.03±0.404c	0.633±0.088c	3.06±0.147b	0.273±0.022bc	3.66±0.33b	7.43±0.448cd	0.0±0.00d	0.0±0.00d
Salt stress with inoculation	3.90±0.360b	1.133±0.120b	3.28±0.339b	0.322±0.015ab	4.00±0.00ab	8.43±0.786bc	68.00±7.54b	98±9.20b
Acid stress without inoculation	2.80±0.366c	0.666±0.033c	3.02±0.148b	0.225±0.020c	3.33±0.333b	6.80±0.503d	0.0±0.00d	0.0±0.00d
Acid stress with inoculation	2.83±0.305c	0.766±0.088c	2.99±0.291b	0.251±0.030b	3.33±0.333b	7.60±0.47bcd	16.33±2.43c	23±4.50c

Table 4. The effect of salt and acid stress on *Triticum aestivum* L. var. DWR 225 inoculated with *Glomus fasciculatum*.

Treatments	Plant height	Stem Diameter	Shoot biomass	Root biomass	Leaf no	Leaf length	% Root colonization	Spore no
Control untreated	4.03±0.152c	0.966±0.208ab	2.906±0.36b	0.224±0.02bc	4.33±0.57ab	9.16±1.04c	0.0±0.00d	0.0±0.00d
Inoculated untreated	6.23±0.208a	1.166±0.152a	5.00±0.264a	0.293±0.03a	5.0±0.00a	15.76±0.208a	79.60±5.98a	140.66±15.94a
Salt stress without inoculation	3.30±0.264d	0.533±0.02d	2.13±0.208c	0.192±0.03d	4.0±0.0b	8.66±0.472c	0.0±0.00d	0.0±0.00d
Salt stress with inoculation	5.36±0.680b	0.900±0.02b	3.26±0.251b	0.230±0.03b	4.66±0.57ab	12.56±0.73b	56.59±5.66b	90.00±6.08b
Acid stress without inoculation	3.10±0.100d	0.666±0.02cd	1.21±0.189d	0.146±0.03e	4.0±0.0b	8.16±0.15c	0.0±0.00d	0.0±0.00d
Acid stress with inoculation	4.80±0.264b	0.833±0.23bc	1.63±0.29d	0.172±0.02de	4.33±0.50b	9.32±0.43c	12.93±0.85c	21.33±5.34c

Discussion

Earlier workers reported as better growth performance of AM fungal inoculated plants to salt and acid stress. Salt resistance was improved by AM fungal colonization in Maize (Al-Karaki *et al.*, 2001). NaCl and HCl treatments were known to reduce Mycorrhizal colonization in Maize (Gupta and Rautaray, 2005). AM fungi were tested to protect Cucumber plants from NaCl stress compared to similar sized non AM plants. Alfalfa was also more effectively protected against salinity stress by AM symbiosis than by P supplementation (Azcon and Barea, 1992) and improvement of NaCl resistance in lettuce plants. Soil salinity affects the crop plants in three ways through osmotic stress, ionic stress and changes in cellular ionic balance, which ultimately decreases the water availability to the host plants resulting in restricting plant growth. Physiologically many processes are affected due to physiological water stress, such as reduced cell growth decreased cell growth, decreased stomatal conductance, decreased photosynthetic rate, decreased biomass and yield. AM

fungi are known to reduce the salt and acid stress and helping the host plants to produce more biomass and yield than non mycorrhizal plants. The mycorrhizal colonization was found to be more in untreated inoculated plants than plants treated with NaCl and HCl high salt concentration may affect mycorrhizal colonization and hyphal growth in plants. Vesicle formation is greatly reduced in stress induced plants in particular in acid treated plants. This is probably because the contents of AM fungi are absorbed by the host plants under stress conditions (Kaspari, 1973). The decrease in the number of spores in the rhizosphere of NaCl treated plants supports the view that vesicles are certainly related to spore formation. Plants treated with acid show poor mycorrhizal colonization, spore and vesicle number. The AM fungus *Glomus fasciculatum* was found to be sensitive to acid stress. However AM fungi are known to increase phosphorus availability in acid soils. AM fungi may increase the uptake of phosphorus and promote growth. This was the reason for better growth of inoculated plants than uninoculated plants (Marschner and Dell, 1994).

Mycorrhizal symbiosis could enhance the plant growth and stress conditions through inducing metabolic changes (Mathur and Vyas, 2000). Reported that mycorrhizal symbiosis resulted in significant increase in protein, chlorophyll, reducing sugars, free amino acids under stress conditions as compared with non mycorrhizal plants. Crude protein content is reported to be higher in mycorrhizal plants than non mycorrhizal plants (Wu and Xia, 2006). AM symbiosis led to enhanced growth, nutrition, productivity and improved yield in Wheat plants (Abo-Ghaila and khalafallah, 2008). Plants colonized by mycorrhizal fungi have shown to absorb water more thoroughly than non mycorrhizal plants (Auge, 2001). This is the reason for higher shoot and root biomass in AM inoculated plants than control plants (Fitter, 1985). It was reported that inoculation with AM fungi brought about an important increase in biomass production which might be attributable to increased dependence of Wheat on AM fungi for water uptake (Al-Karaki *et al.*, 2004). The AM fungus *Glomus fasciculatum* helps the host plants to maintain higher Relative water content than uninoculated plants, thus enabling the mycorrhizal plants to carry out metabolic function even under stress situations without any inhibitory effect of stress (Amerian *et al.*, 2001). Dry weights of AM plants were moderately greater than nonmycorrhizal plants when subjected to salt stress Lakshman and Srinivasulu, 2004).

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