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# MITIGATION OF SALINITY EFFECTS ON *SESBANIA ACULEATA* L., THROUGH ENHANCED AVAILABILITY OF CARBON DIOXIDE

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## Abstract

Experiments were conducted to assess the effect of $CO_2$ on growth and nitrogen fixation in *Sesbania aculeata* L., under different salinity and water regimes. Salinization of soil led to decrease in different growth attributes of the plants. The negative effect of salinity was mitigated to a significant extent at elevated $CO_2$. Growth and N content of the plants was better when grown at 25 than 15% soil moisture level. Roots of *Sesbania* were densely nodulated, the weight of nodules being reduced with the increase in salinity but more at higher moisture level. The analysis of shoot material for total N and $^{15}N$ showed significant amounts of $N_2$ being fixed by the plants. The shoot contained 23-67% of the N from fixation under different growth conditions and 33-77% from the soil. Contribution of biological fixed N to the total N content of shoots decreased with salinity but was more at higher moisture in all the cases. Enclosure of plants in the polyethylene chamber led to an increase in the contribution of biologically fixed N to the total N of shoots irrespective of the soil conditions. Soil salinity had in general a depressing effect on contribution of biologically fixed N suggesting a decrease in the rhizobial activity vis-à-vis decreased photosynthate supply to the root-zone. Significantly higher amounts of N seemed to have been fixed at 25% than 15% soil moisture. Elevated $CO_2$ favoured $N_2$ fixation leading to a greater contribution of fixed N to the total plant N.

## Introduction

Carbon dioxide ($CO_2$) is basis of all life on earth because of being a primary raw material that provided the first molecular link between atmosphere and the biosphere and is therefore more pivotal for the functioning of ecosystems than any other substance. For the past two millennia, the $CO_2$ content of the earth's atmosphere has been fairly constant averaging about 280 ppm. However, with rapid industrialization that coincided with the increase in fossil fuel burning, the $CO_2$ concentration of the air started increasing and is estimated to double by the end of present century (King *et al*., 1992). A vast body of information suggests that elevated $CO_2$ has a beneficial effect on the process of photosynthesis that translates into higher biomass yield and related phenomena (Drake & Leadley, 1991; Idso & Idso, 1994; Azam & Farooq, 2001). This effect is particularly significant for plants having $C_3$ photosynthetic pathway (Akita & Tanaka, 1973) and more so when they are faced with biotic and abiotic stresses imposed both by atmosphere and the rhizosphere (Koch & Monney, 1996).

Amongst different plant types with $C_3$ photosynthetic system, legumes occupy an important position with respect to the functioning of natural and artificial ecosystems through N acquisition from the atmosphere (Zanetti *et al*., 1997; Hopkins, 1999). Since $N_2$ fixation is an energy intensive process, any factor that enhances the supply of carbonaceous substances to the roots will have an enhancing effect on the process. Hence, it is logical that elevated $CO_2$ is reported to have a positive effect on $N_2$ fixation vis-à-vis enhanced rate of photosynthesis and rhizodeposition (Tschaplinski *et al*., 1993; Polley *et al*., 1996; Drake *et al.,* 1997; Hungate *et al*., 1997).

In agroecosystems, green manuring legumes occupy a key position in maintaining/improving soil fertility and productivity. Their significance has increased further as a source of supplemental nitrogen due to economic and pollution potential concerns of chemical N fertilizers. Of the different legumes, species of sesbania have generally been considered as most important for green manuring especially in wheat-rice rotations system. In arid regions, this system is characterized with varying degrees of salinity and water stress. However, there are hardly any studies to demonstrate the impact of elevated $CO_2$ on green manuring legumes especially sesbania raised under these conditions. Therefore, our objective was to study under greenhouse conditions the response of *Sesbania aculeata* grown at different salinity and moisture regimes to elevated $CO_2$.

## Materials and Methods

Soil used in the experiment was collected from the top 0-15 cm of the experimental fields at the Nuclear Institute for Agricultural and Biology, (NIAB), Faisalabad. Air-dried and sieved (< 2mm) soil had the following characteristics: organic C, 0.6%; total N, 0.055%; $NH_4$-N, 7 µg $g^{-1}$ soil; $NO_3+NO_2$-N, 12 µg $g^{-1}$ soil; pH (1:1, soil: water suspension), 7.7; electrical conductivity, 4.65dS$m^{-1}$; sand, 59.6%; silt, 26.2%; and clay, 14.2%.

Five-kg portions of air-dried and sieved soil were filled in 6-kg plastic pots in which *Sesbania* @ 5 seeds $pot^{-1}$ were sown. The stand was thinned to 3 seedlings within 2 days after seed germination. The soil was irrigated with water or water containing NaCl to achieve the following variation in water and salinity regimes:

T1: soil moisture content of 15% (v/w)
T2: soil moisture content of 25% (v/w)
T3: soil moisture content of 15%, NaCl concentration in soil of 450 mg $kg^{-1}$
T4: soil moisture content of 15%, NaCl concentration in soil of 900 mg $kg^{-1}$
T5: soil moisture content of 25%, NaCl concentration in soil of 450 mg $kg^{-1}$
T6: soil moisture content of 15%, NaCl concentration in soil of 900 mg $kg^{-1}$

Nine pots were used for each treatment with triplicate being placed in i) the greenhouse under ambient conditions, ii) polyethylene enclosure and iii) polyethylene enclosure with the provision to increase $CO_2$ level. The polyethylene enclosure consisted of an iron-bar frame (1 x 1 x 1.5 m) covered with polyethylene sheet and having an opening on the top to allow for gaseous exchange. The level of $CO_2$ was raised to approximately twice the natural (370 µmol $mol^{-1}$) by decomposing (through microbial action) sufficient quantities of sucrose added to unplanted soil in separate containers. For this purpose, required amount of sucrose was dissolved in water and applied to soil contained in 300-ml plastic containers such that 15% moisture level was achieved. Sufficient number of containers were used to ensure uniform distribution within the canopy. It was assumed that 50% of the sucrose C would be released as $CO_2$ through microbial respiration within 2 days and added to the ambient $CO_2$ level in the canopy. This assumption is supported by studies where $CO_2$ evolution has been studied using isotopic techniques (Haider & Azam 1982; Azam *et al*., 1985). Every week, the containers with sucrose were replaced with a fresh set to roughly maintain an elevated level of $CO_2$. Irrigations were scheduled weekly when sucrose containers were replaced. However, during the period of excessive loss of water through evapo-transpiration, extra irrigations were given through the opening in canopy top using a rubber pipe. The amount of required irrigation water was determined by weighing the pots receiving no additional $CO_2$.

The gas chromatography of the air samples collected in air-tight medical syringes showed concentration of $CO_2$ in the chamber (having arrangements for elevated $CO_2$) to vary between 356 µmol $mol^{-1}$ and 720 µmol $mol^{-1}$ at different sampling intervals during the plant growth. For maintaining moisture content of the soil at required level, appropriate amounts of water were delivered from the open top of the chambers with the help of a hosepipe. Humidity and temperature inside and outside the chambers was monitored.

Plants harvested 6 weeks after seed sowing were partitioned into root and shoot portions, the nodules separated from the roots and the dry weight (70 $^{o}$C) of the three components recorded. Aliquots of finely powdered root and shoot portions were analyzed for total N using micro-Kjeldahl method (Bremner, 1996). The distillates from shoot samples were further processed for the determination of isotopic ratios using double inlet mass spectrometer as described earlier (Azam, 1990). Calculations for nitrogen derived from fixation were made on the basis of $^{15}$N dilution resulting from $N_2$ fixation. It is evident that soil N has a higher percentage of $^{15}$N relative to the atmospheric N (Azam & Farooq, 2003). Hence, the extent of $N_2$ fixation can easily be determined by exploiting this difference and using the equations given below. Atom % $^{15}$N excess is the difference in $^{15}$N abundance of soil N minus that of atmospheric N i.e., 0.367 which is also termed as natural abundance. A higher rate/extent of $N_2$ fixation will mean a greater dilution of $^{15}$N and thus a lower $^{15}$N excess. Soil used in the present study had an atom % $^{15}$N of 0.3801 and thus an excess of 0.0131.

$$\%\text{ N derived from soil} = \frac{\text{Atom \% }^{15}\text{N excess of plant N}}{\text{Atom \% }^{15}\text{N excess of soil N}} \times 100$$

Statistical analyses of the data included determination of standard deviation of the means using MS EXCEL computer software programme.

## Results and Discussion

Salinity and waterlogging (and recently drought) have been serious problems limiting agricultural production in Pakistan and elsewhere. A review of literature given in the introduction section reveals that elevated $CO_2$ in the atmosphere mitigates the negative effects of these and other environmental stresses imposed on plants as described earlier. In the present study, response of *S. aculeata* to elevated $CO_2$ was investigated under different salinity and water regimes. Salinization of soil had generally a depressing effect on the growth and biomass production of *Sesbania* (Table 1). Biomass of root, shoot and nodules decreased with the salinity under all growth conditions. In spite of being moderately tolerant to salinity, growth reduction at increasing salt levels has been reported in *Sesbania* (Mahmood, 1998). Generic and species differences in salt tolerance have also been reported (Salim *et al*., 1979; Niazi, 1992). These differences are generally attributed to variations in tolerance to ions like $Na^+$ in the root-zone. However, mineralization and availability to plants of essential nutrients, especially N, is affected significantly in the presence of salts (Laura, 1977; Malik & Azam, 1979) thereby affecting plant growth. This will be particularly true for legumes that depend to a significant extent on the N mineralized from soil organic matter that may meet up to 70%

of the plant N requirements. Hence, any factor (salinity in the present studies) that retards the release of N from soil organic matter will have a negative effect on plant growth. Significance of N in determining the plant growth is evident from its close relationship with the root and shoot biomass, essentially similar trends being observed under different growth conditions (Table 2). As a result, significant positive correlation was obtained between the two parameters (N content and biomass yield); co-efficient of correlations for shoot, root and shoot + root being 0.77, 0.99 and 0.94, respectively, when entire data set irrespective of the growth conditions was used for computation ($n = 18$ in each case). Significant correlations between the dry matter yield and N content have been reported by Azam *et al*., 1991.

Higher moisture level in the root-zone had a positive effect on different growth parameters as well as N content of plants (Table 1 and 2). This could be attributed to enhanced $N_2$ fixation under high moisture conditions that limit the availability of oxygen at the nitrogenase level; a condition suitable for efficient $N_2$ fixation (Sangakkara *et al*., 1996). Since legumes depend mainly on biological $N_2$ fixation besides N from soil organic matter, the factors that support the former process will have a positive impact on plant growth. The negative effect of salinity also appeared to be mitigated by higher moisture level probably because of dilution of salts in the soil solution. This would also result in better plant growth especially at higher salinity level.

In the present study one of the main objectives was to determine the effect of elevated $CO_2$ on growth performance of *Sesbania*. Plants grown in polyethylene chamber with elevated $CO_2$ showed better growth performance. It is frequently reported that elevated $CO_2$ favours different physiological processes of the plants thereby leading to increased biomass production and ecosystem functioning (Drake & Leadley, 1991; Idso & Idso, 1994; Azam & Farooq, 2001). The effect is more pronounced for plants facing stresses imposed through soil or atmosphere (Koch & Monney, 1996). Polley *et al.* (1996) reported increased survival of seedlings subjected to water stress at elevated $CO_2$. A general response to doubling of $CO_2$ concentration is 30-60% reduction in stomatal conductance and leaf stomatal density. As a result, improvements in water use efficiency have often been reported (Hogan *et al.,* 1991). In fact high $CO_2$ concentration increases the use efficiency of light, nutrients and water (Gunderson & Wullschleger, 1994).

Symbiotic nitrogen fixation in legumes is a fundamental process for maintaining soil fertility and the continued productivity of natural and agroecosystems. Under favourable conditions, legumes can derive as much as 80-90% of their N requirement through $N_2$ fixation (Eaglesham *et al.,* 1977; Rennie *et al.,* 1982). The amounts of N fixed may vary from 57 to 600 kg N $yr^{-1}$ $ha^{-1}$, minimum being for soybean (57 - 94 kg N $yr^{-1}$ $ha^{-1}$) and maximum (128-600 kg N $yr^{-1}$ $ha^{-1}$). In the present study, *Sesbania* roots were found to bear abundant nodules suggesting the presence of effective rhizobia in sufficient numbers. This was possible as the soil was collected from a field previously sown to this crop. Hence, it was possible to determine the contribution of biological $N_2$ fixation to the total N in plants using $^{15}N$ isotopic dilution technique with some modification. In practice, a non-leguminous reference crop is required to estimate $N_2$ fixation in the legume (Azam & Farooq 2003). In the present study, however, the difference in $^{15}N$ abundance of soil N and atmospheric N was exploited to determine percent N derived from soil. Since, no fertilizer N was applied and N derived from soil (Ndfs) could be determined, that derived from the atmosphere (through fixation) was easy to determine by subtraction i.e., 100 – Ndfs. Soil N had a $^{15}N$ abundance of 0.3801 and $^{15}N$ excess of 0.0131 (i.e., 0.3801 – 0.367 which is the natural abundance) and thus could be used to calculate the percentage of N derived from soil and atmosphere.

**Table 1. Dry matter distribution in different plant components of *Sesbania aculeata* as affected by growth conditions*.**

| | **M1** | | | **M2** | | |
|---|---|---|---|---|---|---|
| | **C1** | **C2** | **C3** | **C1** | **C2** | **C3** |
| | | | **Dry weight of shoot (g pot$^{-1}$)** | | | |
| S1 | 4.95±0.32 | 5.26±0.22 | 5.78±0.22 | 5.50±0.19 | 5.82±0.31 | 6.67±0.23 |
| S2 | 4.58±0.41 | 5.11±0.25 | 5.46±0.31 | 5.89±0.23 | 5.88±0.45 | 6.37±0.31 |
| S3 | 4.19±0.28 | 4.63±0.26 | 5.32±0.44 | 5.29±0.15 | 5.56±0.28 | 5.65±0.41 |
| | | | **Dry weight of root (g pot$^{-1}$)** | | | |
| S1 | 3.15±0.11 | 1.78±0.11 | 1.51±0.12 | 3.78±0.07 | 2.86±0.16 | 1.99±0.21 |
| S2 | 2.57±0.12 | 1.82±0.08 | 1.34±0.15 | 3.94±0.12 | 3.39±0.12 | 1.77±0.12 |
| S3 | 2.20±0.09 | 2.31±0.12 | 1.50±0.05 | 2.82±0.09 | 2.97±0.21 | 1.53±0.18 |
| | | | **Dry weight of nodules (g pot$^{-1}$)** | | | |
| S1 | 0.362±0.03 | 0.197±0.02 | 0.311±0.01 | 0.524±0.02 | 0.314±0.03 | 0.477±0.03 |
| S2 | 0.344±0.02 | 0.216±0.02 | 0.309±0.03 | 0.529±0.04 | 0.392±0.05 | 0.437±0.02 |
| S3 | 0.202±0.02 | 0.178±0.03 | 0.288±0.02 | 0.268±0.03 | 0.349±0.03 | 0.419±0.01 |
| | | | **Total dry weight (g pot$^{-1}$)** | | | |
| S1 | 8.46±0.76 | 7.24±0.35 | 7.60±0.51 | 9.80±0.64 | 9.00±1.01 | 9.13±0.78 |
| S2 | 7.50±0.60 | 7.14±0.61 | 7.10±0.49 | 10.35±0.61 | 9.66±0.67 | 8.58±0.63 |
| S3 | 6.59±0.61 | 7.12±0.49 | 7.11±0.81 | 8.65±0.72 | 8.61±0.29 | 7.60±0.69 |

*, C1, in open; C2, in polyethylene chamber at ambient $CO_2$, C3, in polyethylene chamber at elevated $CO_2$; M1, field capacity moisture; M2, twice the field capacity moisture; S1, S2, and S3 are salinity levels i.e., control, 450 mg kg$^{-1}$ and 900 mg kg$^{-1}$ NaCl added to the soil, respectively ± shows standard deviation of the means

**Table 2. N yield and its distribution in root and shoot portions of *Sesbania aculeata* as affected by growth conditions*.**

| | **M1** | | | **M2** | | |
|---|---|---|---|---|---|---|
| | **C1** | **C2** | **C3** | **C1** | **C2** | **C3** |
| | | | **N content of shoot (mg pot$^{-1}$)** | | | |
| S1 | 140.5±6.8 | 166.0±15.2 | 156.7±7.2 | 195.2±16.1 | 184.0±14.2 | 178.9±13.2 |
| S2 | 153.8±7.3 | 160.3±13.2 | 156.5±8.7 | 181.7±13.8 | 177.5±10.2 | 171.7±10.7 |
| S3 | 138.1±8.2 | 132.2±13.8 | 129.9±9.2 | 196.3±20.1 | 161.5±12.1 | 168.6±14.9 |
| | | | **N content of root (mg pot$^{-1}$)** | | | |
| S1 | 47.5±3.2 | 31.9±2.4 | 29.4±3.5 | 54.9±3.6 | 50.5±4.1 | 33.5±2.8 |
| S2 | 39.7±5.1 | 33.6±2.7 | 27.8±1.8 | 55.2±4.2 | 57.7±2.8 | 32.7±2.1 |
| S3 | 34.2±4.2 | 38.3±1.9 | 30.0±2.6 | 45.9±3.7 | 47.0±2.3 | 27.7±3.2 |
| | | | **Total plant N (mg pot$^{-1}$)** | | | |
| S1 | 188.0±14.2 | 197.9±6.2 | 186.1±9.2 | 250.1±18.3 | 234.5±18.2 | 212.4±13.4 |
| S2 | 193.5±12.8 | 193.9±10.1 | 184.3±10.2 | 236.9±21.2 | 235.2±14.7 | 204.4±18.7 |
| S3 | 172.3±13.7 | 170.5±13.2 | 160.0±11.1 | 242.2±20.6 | 208.5±21.4 | 196.3±16.2 |

*, see the footnote for Table 1
± shows standard deviation of the means

Data presented in Table 3 show 53-77% of the plant N being derived from soil and the rest (23-47%) from the atmosphere through biological nitrogen fixation. This percentage is relatively less than that commonly reported, i.e., 80-90% (Eaglesham *et al.,* 1977; Rennie *et al.,* 1982) but possible as the plants were harvested at an early stage of growth. Different factors had important bearing on the process of $N_2$ fixation. Soil salinity had a negative effect while high moisture a substantially positive effect on the contribution of biological $N_2$ fixation to the total plant N. This was attributable to the overall effect of these factors on plant growth. Reduced plant growth with increased salinity would lead to less photosynthesis and partitioning of photosynthates to the soil to

Table 3. Effect of different growth conditions* on plant parameters pertinent to $N_2$ fixation by isotopic dilution

| Dry matter, g pot$^{-1}$ | | N yield, mg pot$^{-1}$ | | $^{15}$N excess | | Ndfs, % | | Ndfa, % | |
|---|---|---|---|---|---|---|---|---|---|
| M1 | M2 | M1 | M2 | M1 | M2 | M1 | M2 | M1 | M2 |
| 95±0.32 | 5.50±0.19 | 140.5±10.1 | 195.2±10.3 | 0.0093 | 0.0053 | 70.99±2.13 | 40.46±3.11 | 29.01±2.11 | 59.54±3.21 |
| 58±0.41 | 5.89±0.23 | 153.8±11.7 | 181.7±12.8 | 0.0095 | 0.0068 | 72.52±4.21 | 51.91±2.23 | 27.48±1.98 | 48.09±3.28 |
| 19±0.28 | 5.29±0.15 | 138.1±9.5 | 196.3±14.8 | 0.0101 | 0.0075 | 77.10±3.76 | 57.25±2.14 | 22.90±2.01 | 42.75±1.95 |
| 26±0.22 | 5.82±0.31 | 166.0±10.5 | 184.0±10.3 | 0.0083 | 0.0055 | 63.36±1.83 | 41.98±3.15 | 36.64±2.76 | 58.02±3.71 |
| 11±0.25 | 5.88±0.45 | 160.3±13.2 | 177.5±13.2 | 0.0079 | 0.0059 | 60.31±3.21 | 45.04±2.22 | 39.69±3.01 | 54.96±1.85 |
| 63±0.26 | 5.56±0.28 | 132.2±10.6 | 161.5±14.6 | 0.0089 | 0.0067 | 67.94±3.02 | 51.15±4.76 | 32.06±1.37 | 48.85±2.02 |
| 78±0.22 | 6.67±0.23 | 156.7±14.2 | 178.9±10.2 | 0.0071 | 0.0048 | 54.20±2.76 | 36.64±2.65 | 45.80±3.12 | 63.36±2.87 |
| 46±0.31 | 6.37±0.31 | 156.5±12.4 | 171.7±13.2 | 0.0069 | 0.0043 | 52.67±3.21 | 32.82±3.12 | 47.33±2.54 | 67.18±3.32 |
| 32±0.44 | 5.65±0.41 | 129.9±7.9 | 168.6±14.6 | 0.0073 | 0.005 | 55.73±2.78 | 38.17±1.87 | 44.27±2.87 | 61.83±2.92 |

e footnote for Table 1

standard deviation of the means

support $N_2$ fixation at higher salinity level. Enhanced rates of photosynthesis, on the other hand, should allow greater partitioning of carbohydrates below-ground, thereby enhancing root growth and enabling plants to better explore the soil for water and nutrients (Wall, 2001). In the present study, high moisture promoted the contribution of $N_2$ fixation to N nutrition of plants. This is possible as the higher soil moisture is normally inhibitory to N mineralization from soil organic matter and hence $N_2$ fixation was the predominant source of N in plants grown at this moisture level. Decrease in soil N mineralization at higher salinity and moisture levels has been well-documented (Laura, 1977; Malik & Azam, 1979).

Contribution of BNF (biological nitrogen fixation) decreased with salinity, but elevated $CO_2$ arrested the decrease to a significant extent. Such benefits are reported to be derived from the availability of more solutes for osmoregulation, from reduction in the transpirational intake of salts (Bazzaz, 1990), or by improving RUBISCO activity. Munns *et al*., (1999) observed $CO_2$ and salinity interaction at low but not at high salinity. In $C_4$ plants elevated $CO_2$ helps overcome the leakiness induced by salt stress (Wong & Osmond, 1991). In addition, positive effect of elevated $CO_2$ on root proliferation and root-induced microbiological and biochemical changes may help plants withstand salinity stress.

The observations recorded in the present study are in conformity with those of Yu *et al*., (2002) who showed stimulation of symbiotic $N_2$ fixation at higher levels of $CO_2$. Zanetti *et al*., (1997) reported that the total N yield increased consistently and the percentage of plant N derived from symbiotic $N_2$ fixation increased significantly in *Trifolium repens* under elevated $CO_2$. This increase is often attributed to increased C availability for nodule formation and for the functioning of nitrogenase that is responsible for reduction of $N_2$ (Tissue *et al*., 1997; Serraj *et al*., 1998). Partitioning of photosynthates to roots is reported to increase at elevated $CO_2$ levels (Tschaplinski *et al*., 1993; Hungate *et al*., 1997) and would help legumes perform better under stress situations (Tingey *et al*., 2000). Elevated $CO_2$ directly stimulates the activities of microbes which are responsible for nitrogen fixation present in all natural ecosystems (Lowe & Evans, 1962). An increase in nodule mass of *S. aculeata* observed in the present study also conforms to the reports showing higher nodule mass due to transport of saccharides from the shoot to roots, which is known to down-regulate the rate of photosynthesis in plants grown at elevated $CO_2$ (Drake *et al*., 1997). At elevated $CO_2$, Murillay *et al*., (1999) observed increased population of rhizobia associated with the root of white clover. Luscher *et al*., (1998) exposed several different types of grassland species to elevated atmospheric $CO_2$ concentration and observed that $N_2$ fixing species tended to produce more biomass than the non-fixing species. Dakora & Drake (2000) found that at 300 $\mu$mol mol$^{-1}$ increase of $CO_2$ content in air actually increased nitrogenase activity in $C_3$ and $C_4$ species by 35% and 13%, respectively. In studies using several kinds of plants, Luscher *et al*., (1998) found legumes to be the most responsive to elevated $CO_2$. Amthor *et al*., (1994) and Reeves *et al*., (1994) observed significant increase in different yield parameters of soybean.

Results of this study suggest a positive effect of elevated $CO_2$ on growth and $N_2$ fixation of *S. aculeata* grown under mild salinity and high moisture. It is possible, therefore, to enhance the biomass yield of this green manuring crop by elevating the level of $CO_2$ in the plant canopy. Under practical agricultural conditions, plant residues decomposing on the soil surface following mulching may help elevate the level of $CO_2$ and thus the plant growth.

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