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Potential of Plant Growth Promoting Rhizobacteria for Improvement of Growth and Biochemical Status of Wheat Seedlings under Salinity

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ABSTRACT: Wheat is a staple and most widely grown crop in the world; it holds the first rank in the world among grain production. As the population expands exponentially, the consumption and production rate of wheat also increase. For higher production, various chemical fertilizers are used in an uncontrolled manner. Hence, these practices affect both wheat yield and soil health, specifically salinity. In this study, improvement of growth wheat under saline condition through microbe-plant interaction was evaluated. For this purpose, a bacterial isolated from rice rhizosphere i.e. PT-5A having multiple plant growth promoting traits was interacted with wheat seedlings under normal as well as saline condition. This isolate demonstrated relative growth up to 36.52% in 25% NaCl salt concentration compared to growth in non-saline condition. In plant-microbe interaction experiment, wheat seedlings grown in hydroponic condition exhibited better growth parameters when inoculated with isolate PT-5A under normal and saline condition. Bacterial isolate PT-5A also eliminated salt stress through improvement of biochemical and antioxidant status of seedlings. It improved membrane stability of the seedlings as determined by electrical conductivity and malondialdehyde equivalent quantification. In salt stress, PGPR strain PT-5A inoculated seedlings showed a significantly increased chlorophyll content and decrease in proline, H_2O_2 content, SOD and POD activities, malondialdehyde concentration, and TSS content in comparison to the negative control seedlings. This research concludes that inoculating wheat seedlings with PGPR stain PT-5A improved their biochemical and physiological parameters and helped the wheat seedlings to extreme salt stress conditions.

Keywords: Wheat, Salt tolerance, PGPR, Biochemical, Morphological**.**

INTRODUCTION

Wheat (*Triticum aestivum*) is a major crop of Gramineae family and considered the most significant grain crop among all the cereals. It ranks first globally among grain-producing crops, especially for human consumption (Giraldo *et al.*, 2019). China and India are projected to be the world's second and third-largest producers of wheat in 2023, respectively, with output in those nations mainly inaccessible to the global market. Global output is rising, which encourages higher commerce, more consumption, and larger ending stockpiles. In the race for high yield production, soil is extremely affected and turns into unhealthy and sick soil. Due to this, the rate of production and productivity both decrease day by day, and the actual cause of this is soil salinity. Salt stress affects 20% of global cultivable land and is increasing continuously owing to the change in climate and anthropogenic activities (Arora, 2019). Soil salinization may occur for both natural and anthropogenic reasons. Out of 932.2 million ha of salt-affected soils worldwide, the extent of human-induced salinization is 76.6 million ha (Oldeman *et al.*, 1991; Mashali, 1995; Shahid *et al.*, 2018). According to Mandal *et al.* (2018), more recent estimates show an increasing trend in the global salt-affected area, with an area of 1,128 million ha. It is estimate that, 20% of total cultivated and 33% of irrigated agricultural lands worldwide are afflicted by high salinity (Shrivasata and Kumar 2015). Around 6.727 million ha of area in India, which is around 2.1% of the geographical area of the country, is salt-affected, of which 2.956 million ha are saline and the rest 3.771 million ha are sodic (Arora *et al.*, 2016; Arora and Sharma 2017). Nearly 75% of salt-affected soils in the country exist in the states of Gujarat (2.23 million ha), Uttar Pradesh (1.37 million ha), and Maharashtra (0.61 million ha).

Wheat is more susceptible to salinity than other field crops, which inhibits plant growth and development, results in low production, or even causes crop failure under extremely severe salinity. At 6 to 8 dS m^{-1} of salt stress, wheat crop production begins to decrease. Under salt stress, the production of ROS from Na^+ toxicity damages biomolecules at the cellular level and changes redox homeostasis (Kundu *et al.*, 2018).

Salinity reduces seedling development, dispersal of germination events, and seedling metabolism, which results in slower plant growth and lower agricultural yield (El Sabagh *et al.*, 2019a&b; El Sabagh *et al.*, 2020). Additionally, salt stress causes oxidative stress and affects the intake of water and nutrients, hormone balance, photosynthesis, membrane structure, and enzyme activity (Taha *et al.*, 2021; Ibrahimova *et al.*, 202l; Seleiman *et al.*, 2021a). Salinity has a detrimental impact on wheat phenological changes, including leaf number, leaf growth rate, root/shoot ratio, and biomass output (El-Hendawy *et al.*, 2005). Salinity stress accelerates all phenological phases of wheat (Grieve *et al.*, 1994), decreases the number of fertile tillers (Abbas *et al.*, 2013), decreases the number of spikelets (Frank *et al.*, 1987), decreases kernel weight (Abbas *et al.*, 2013), and negatively impacts grain yield (Sorour *et al.*, 2019). Wheat under salt stress, for example, has seen yield reductions of up to 45% (Ali *et al.*, 2009).

Microbial inoculation to relieve salt stress is a superior solution since it reduces production costs and environmental risks (Sen and Chandrasekhar 2014). Plant growth promoting rhizobacteria (PGPR) has been shown to be effective in agriculture. It has also been hypothesized that their growth-promoting effects under stressful situations are advantageous for crop production. Various researchers have demonstrated alleviation of effect caused by salinity in different crops as well as wheat. With the use of halotolarent bacteria with plant growth promoting traits were able to mitigate salt stress in wheat (Kerbab *et al.*, 2021). The effect of PGPR inoculation in wheat on various growth, biochemical and physiological parameters under NaCl stress has been assessed by many researchers (Ilyas *et al.*, 2020; Safdarian *et al.*, 2020; Babar *et al.*, 2021; Ayaz *et al.*, 2022).

In this study, multiple PGPR isolates were studied isolated from the rhizosphere of rice from different regions. Among these bacteria, various isolates exhibited direct and indirect plant growth-promoting traits like indole-3-acetic acid (IAA) production, phosphate, zinc and potassium solubilization and volatile production. Hence, the experiment was conducted to analyze the impact PGPR isolate on wheat seedlings to alleviate salt stress.

MATERIAL AND METHODS

A. Assessment of NaCl tolerance of PGPR isolate and inoculum preparation

The PGPR strain, PT-5A was previously isolated from the rhizosphere of rice plants (data not published). These isolate showed a variety of plant growth-promoting (PGP) characteristics, including the solubilization of zinc, phosphate, and potassium as well as production of NH_3, indole acetic acid (IAA), and the 1-carboxylic acid (ACC) enzyme. To assess the growth of isolate under salinity, overnight grown culture was inoculated in Luria Bertani broth (LB) medium supplemented with several doses of NaCl (0, 5%, 10%, 15%, 20%, and 25% (w/v)). Using a UV-vis spectrophotometer (V550, Jasco, Japan) at 600 nm, the optical density of the bacterial cultures was determined.

For preparation of inoculum for plant microbe interaction experiment, method described by Gontia-Mishra *et al.* (2016) was used. Overnight grown culture of isolate PT-5A centrifuged at 5000 rpm for 5 min and suspended in 0.5Mm MS medium (Murashige and Skoog, 1962) with maintaining an optical density of 0.6 at 600 nm (10^8 colony forming unit).

B. Screening of NaCl tolerance in Wheat

Seeds of the wheat variety GW 322 were obtained from Breeder seed production unit, JNKVV, Jabalpur. The healthy seedlings were chosen, sterilized using 3% sodium hypochlorite solution (v/v) for 10 min followed by three washes with sterile distilled water. Wheat seeds were grown hydroponically in ½ XMS medium with varying concentrations of NaCl (0, 50, 100, 150, and 200 mM) to test the susceptibility of the seedlings to the salt. In final experiment, 100 mM NaCl concentration was used because plants had nominal inhibitory effect on seedlings, which could be overcome by the use of PGPR that is NaCl resistant.

C. Inoculation of PGPR and NaCl stress Treatment

Experiment was conducted as per the method described by Gontia Mishra *et al.* (2016a). Wheat seedlings were sterilized before being put on plastic cups and net for hydroponic growth using a half MS medium with or without 100 mM NaCl treatment. The seedlings without bacterial isolate and 100 mM NaCl treatment served as the positive control, while the uninoculated seedlings with 100 mM NaCl served as the negative control. There were two different types of treatments planned, seedlings inoculated with the PGPR strains PT-5A, without 100 mM NaCl treatment, and seedlings inoculated with the same strains but with the addition of 100 mM NaCl. Each experiment was carried out in triplicate. The leaves and roots samples were obtained for various studies after the treatment period of 10 days.

D. Morphological analysis

After 10 days of treatment, different plant growth parameters including root and shoot length, dry shoot and root weight (DW), and fresh shoot and root weight (FW) were measured. Relative water content (RWC) was calculated using the formula given by Zhang and Blumwald (2001).

E. Biochemical analysis

Chlorophyll content was analyzed according to Arnon (1949). According to Bates *et al.* (1973), the proline content in the leaves was determined. Method prescribed by Velikova *et al.* (2000) was used to determine H_2O_2 concentration in the leaves. Anthrone reagent method was used to estimate Total soluble sugar (Shukla *et al.*, 2012). The protein concentration was measured using the Bradford test (Bradford, 1976). Malondialdehyde (MDA) was the principal reactive metabolite used to quantify the amount of lipid peroxidation in plant tissues Hodges *et al.* (1999). According to Lutts *et al.* (1996) methodology, the electrolyte loss of the leaf tissues was evaluated.

F. Superoxide dismutase (SOD) and peroxidase (POD) activity

According to Rao *et al*. (1996) approach, the POD activity was assayed using guaiacol as the substrate and quantified by guaiacol's oxidation at 470 nm by H_2O_2.The measurement of SOD activity followed Sharma *et al.* (2010). The absorbance was measured at 560 nm, and one unit of SOD activity was defined as the quantity of enzyme that prevented 50% of the degradation of the dye nitro-blue tertazolium (NBT).

G. Statistical analysis

For each trial, information was gathered from 10 wheat seedlings. The mean values and standard deviations of each experiment were computed after three repetitions. Using Web Agri Stat Package (WASP), which is accessible at http,//www.ccari.res.in, a single factor ANOVA analysis was performed for NaCl tolerance (100 mM) and bacterial treatment. To determine the differences that were statistically significant between the means of the bacterial treatment under control and 100 mM NaCl stress, the critical difference (C.D.) values were computed at the 0.05 level. Different letters are used to show the values that are significantly different.

RESULTS

A. Effect of NaCl on Isolate PT-5A

As results demonstrated in Fig. 1, the isolate PT-5A showed significant growth upto 25% of NaCl in LB broth. However, a significant reduction in growth of isolate was noted as concentration of NaCl was increased in the broth. As compare to control, 36.52% of relative growth was observed at 25% NaCl in the broth.

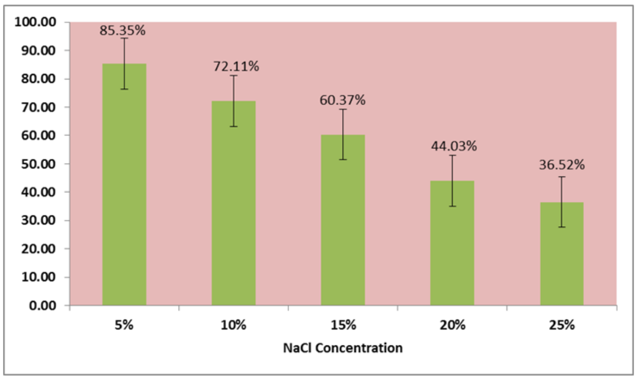

Fig. 1. Relative growth of isolate PT-5A under different salt concentration

B. Effect of isolate PT-5A on the growth of wheat seedlings under normal and saline condition

By cultivating wheat seedlings on ½ X MS medium supplemented with 100 mM NaCl concentration, the ability of PGPR strain PT-5A to mitigate the harmful effects of NaCl on the plants was examined. The length of shoot and root, fresh and dry weight of shoot and root were significantly higher in PGPR inoculated plants when compared with positive control plants under normal condition. The salt condition significantly reduced all the growth parameters compared to plants of positive control and PT-5A inoculated seedlings (Table 1).

The effect of salinity was found reduced in PT-5A inoculated seedlings as demonstrated by enhancement in all growth parameters. There was visible difference in shoot and root lengths caused by normal and saline condition as well enhancement of shoot and root length by inoculation of isolate PT-5A (Fig. 2).

C. Influence of PGPR strain PT-5A on biochemical parameters of wheat seedlings

By assessing various plant biochemical parameters, the impact of NaCl stress on wheat seedlings in the presence of NaCl-tolerant PGPR was investigated (Table 2). Compared to positive control seedlings and seedlings treated with PGPR strain PT-5A, the chlorophyll and protein content of negative control seedlings was much lower. Under NaCl stress, the PGPR strain PT-5A inoculated seedlings showed a significantly increased chlorophyll content than the negative control seedlings. Proline and TSS contents are the biochemical markers of NaCl stress. Inoculation with PT-5A strain improved the protein content in the shoots of wheat seedlings under NaCl stressed conditions. The highest level proline and TSS content was noted in uninoculated seedlings exposed to salt stress. PGPR isolate PT-5A inoculated seedlings under NaCl stress condition showed significant reduction in proline and TSS content in comparison to negative control seedlings. NaCl stress is biochemically indicated by proline and TSS contents. Under NaCl stress, wheat seedlings' protein content in their shoots increased after being inoculated with the PT-5A strain. The uninoculated seedlings subjected to salt stress exhibited the highest levels of proline and TSS content. When compared to negative control seedlings, PGPR isolate PT-5A inoculated seedlings under NaCl stress conditions exhibited a considerable reduction in proline and TSS content.

Fig. 2. Effect of salt stress on morphology of wheat seedlings. A. Positive control, B. Negative control, C. PT-5A, D. PT-5A+ 100mM NaCl.

D. Measurement of oxidative stress and antioxidant enzyme activities

The amount of H_2O_2 in wheat seedlings under salt stress was noticeably higher. H_2O_2 concentration was significantly reduced in their leaves after being inoculated with PGPR isolate PT-5A under salt stress in wheat seedlings. When PGPR-inoculated wheat seedlings under non-stress conditions were compared to positive control seedlings, the seedlings under salinity stress demonstrated significantly higher SOD and POD activity. Under NaCl stress, the amount of malondialdehyde in the roots and shoots was measured. In comparison to negative control seedlings, the shoots of PGPR isolate PT-5A inoculated and positive control seedlings had a substantially reduced level of malondialdehyde. Comparing the roots of PGPR isolate PT-5A inoculated and positive control seedlings to those of negative control seedlings, a noteworthy decrease in malondialdehyde concentration was observed (Table 2).

On the other hand, PGPR isolate PT-5A inoculated seedlings showed a much lower level of malondialdehyde than negative control seedlings under NaCl stress conditions. When seedlings growing under NaCl conditions were tested for EL leakage, similar patterns of results were observed. When compared to the positive control and PGPR isolate PT-5A infected seedling under normal and NaCl conditions, the EL leakage was greater in the negative control plants. Table 2 illustrates how PGPR isolate PT-5A, which is tolerant of NaCl affects electrolyte leakage and malondialdehyde levels in wheat seedlings grown in both salt stress and control environments.

DISCUSSION

Salinity directly affects the physio-chemical and biological characteristics of soil, which can have negative consequences on plant productivity and growth. Plants feeling salinity stress exhibit deprived growth as it causes ionic imbalance and disrupts the proper uptake of nutrients. Osmotic stress, particular ion toxicity, nutritional imbalances, and/or a combination of these variables cause salt to negatively impact plant growth. Numerous studies have reported using PGPR inoculation to reduce salt stress in a variety of crops (Shukla *et al.,* 2012; Bharti *et al.,* 2016; Sapre *et al.,* 2018; Sapre *et al.,* 2022). Our findings showed that the inoculation of PGPR that is tolerant of NaCl enhanced plant development in both non-stressful and salinity-stressed conditions. This implied that the NaCl stress was lessened by the interaction with rhizobacteria.

Under normal condition, PGPR inoculated seedlings demonstrated enhanced growth compare to positive control. Plant growth promotion features such IAA production, ACC deaminase activity, volatile compound generation, and mineral nutrient solubilization are responsible for the enhancement of wheat seedlings' overall growth parameters as these all attributes were demonstrated by PGPR strain. When exposed to salt, the roots of the PGPR-inoculated seedlings were longer than those of the untreated seedlings. This is explained by the fact that PGPRs can generate IAA in saline stress conditions and non-stressed conditions. It is commonly known that rhizobacteria produce phytohormones, particularly IAA, which are involved in root initiation and the elongation of adventitious and lateral roots. This helps the host plant absorb nutrients to the maximum extent possible (Patten and Glick 2002; Jha *et al.,* 2012). Similar results were demonstrated by Sapre *et al.* (2018) in oat as well as in wheat (Gontia-Mishra *et al.,* 2017). The PGPR that was utilized to inoculate the wheat seedlings also exhibits ACC deaminase activity in both salt stress and normal conditions, which indirectly reduces the generation of ethylene during salinity stress and promotes the development of a longer root system. According to publications (Ali *et al.,* 2014; Win *et al.,* 2018; Sarkar *et al.*, 2018; Sapre *et al.*, 2019), salinity stress can be reduced by applying PGPR in conjunction with ACC deaminase activity.

According to Sapre *et al.* (2018), a plant's chlorophyll content is directly correlated with photosynthetic activity and tends to decrease under salinity stress. In the negative control wheat seedlings, the relationship between the concentration of NaCl and the amount of chlorophyll was inverse. The use of PGPR under both salinity levels increased the reduction in chlorophyll content. When wheat seedlings were inoculated with bacterial isolate PT-5A, the increase in chlorophyll content was observed under saline condition. Our findings are consistent with studies that show increased chlorophyll content during salt stress due to PGPR inoculation (Ali *et al.,* 2014; Vimal *et al.,* 2019). Important biochemical indicators of a plant's ability to withstand salt include proline and total soluble sugars (Ashraf and Harris 2004). According to Sandhya *et al.* (2010), double sugars are osmolytes that help with osmotic changes under various abiotic stressors. Both PGPR-inoculated and un-inoculated wheat seedlings showed a substantial rise in TSS levels during NaCl stress (Table 2).

Under NaCl stress, the uninoculated wheat seedlings displayed a lower protein content. It is commonly known that increased formation of Reactive Oxygen Species (ROS) occurs during salt stress, leading to increased oxidative stress and, ultimately, lower protein content as a result of sustained oxidative damage. During NaCl stress, the wheat seedlings inoculated with PGPR exhibited notably elevated protein content (Table 2). Analogous outcomes regarding the rise in protein content in groundnut plants treated with PGPR under salinity stress were shown by Kandasamy et al. (2009), Shukla *et al.* (2012), and Sapre *et al.* (2018).

Reactive oxygen species (ROS) are produced in smaller quantities by plants throughout their metabolic processes in non-saline environments (Abbas *et al.,* 2019). Plants under salinity stress produce reactive oxygen species (ROS). According to Kang *et al.* (2014), ROS interacts with the macromolecules in plants to cause oxidative damage to proteins, lipids, and nucleic acids. According to Egamberdieva *et al.* (2017), ROS also cause damage to the cell membrane through the peroxidation of membrane lipids, which causes electrolytes to seep from the membrane. The application of PGPR to wheat seedlings under salt stress resulted in significantly reduced electrolyte leakage from shoot tissue. This suggests that PGPR strengthened the integrity of the cell membrane against the detrimental effects of salinity.

In the present investigation, wheat seedlings exposed to salinity stress demonstrated a noteworthy rise in H_2O_2 content, while seedlings inoculated with PGPR demonstrated a noteworthy reduction in H_2O_2 content. Our findings concur with those of Zhu *et al.* (2004); Sapre *et al.* (2018), who found that PGPR inoculation under stressful settings decreased the amount of H_2O_2. Plants have evolved an antioxidant defense system that comprises antioxidant enzymes like catalase, SOD, and POD to protect against oxidative damage caused by ROS (Miller *et al.*, 2010). Similarly, in saline conditions, PGPR inoculation with PT-5A resulted in a discernible decrease in the SOD and POD activities of wheat seedlings compared to un-inoculated seedlings. Our data are consistent with those obtained from the beneficial PGPR inoculation of cucumber and oat seedlings, which decreases antioxidant enzyme activity to lessen the effects of salt stress (Kang *et al.,* 2014; Sapre *et al.,* 2018). Therefore, it was expected that, in comparison to uninoculated wheat seedlings, PGPR-inoculated seedlings would be able to withstand the effects of salt stress and, as a result, show reduced levels of antioxidant enzymes.

Lipid peroxidation caused by oxidative stress damages the cell membrane, altering its permeability and increasing electrolyte leakage from the cells (Shukla *et al.,* 2012). As a result, the electrolyte that builds up inside the membrane seeps into the tissues around it (Zhou *et al.,* 2009). Wheat seedlings inoculated with PGPR isolate PT-5A under salt stress showed noticeably reduced electrolyte leakage from shoot tissue, indicating that PGPR shielded the integrity of the plant cell membrane from NaCl damaging effects (Table 2). MDA is one of the compounds produced when ROS oxidize unsaturated fatty acids (Bharti *et al.*, 2014). Owing to oxidative stress, malondialdehyde concentration may be a sign of oxidative membrane damage. According to previous research protecting plants from oxidative damage brought on by salt stress, the reduced levels of malondialdehyde in bacterized plants under stress are consistent (Bharti *et al.,* 2014; Barnawal *et al.,* 2014).

Table 1: Effect of isolate PT-5A on various growth parameters of wheat seedlings under control and saline condition (in 10 days old seedlings).

Treatment	Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg)	Root fresh weight (mg)	Shoot dry weight (mg)	Root dry weight (mg)
Positive control	15.33 ±0.06	7.37 ±0.21	81.4 ±1.0	22.27 ±0.70	33.67 ±0.7	7.67±0.5
Negative control	10.70 ±0.46	4.90 ±0.10	59.9 ±0.9	11.67 ±1.17	27.87 ±1.6	4.40±0.6
PT-5A	18.07 ±0.38	8.73 ±0.15	92.6 ±1.0	24.07 ±1.36	37.73 ±1.7	8.07±0.5
PT-5A + 100mM NaCl	13.13 ±0.59	6.80 ±0.30	75.1 ±0.7	15.80 ±1.31	31.73 ±1.1	5.87±0.7

Data are given as mean ± standard deviation of three replicates; means followed by same letters are not significant at 5% level

Table 2: Effect of isolate PT-5A on various biochemical parameters of leaf tissue under control and saline condition (in 10 days old seedling).

Treatment	Proline (µg g^{-1} FW)	Total soluble sugar (mg g^{-1} FW)	Total soluble Protein (µg g^{-1} FW)	MDA equivalent (nmol g^{-1} FW)	H_2O_2 (mM g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	SOD (U/mg FW)	POD (µM/ g^{-1} FW Min^{-1})	Electrolyte leakage (%)
Positive control	30.85±1.99	1.87±0.19	278.2±2.48	34.23±1.4	3.61±0.9	0.44±0.04	0.164±0.18	2.4±0.19	41.19±1.3
Negative control	43.51±1.88	4.20±0.11	211.7±2.74	64.97±2.1	9.23±1.1	0.31±0.06	0.389±0.13	8.9±0.13	63.87±2.4
PT-5A	15.71±1.23	2.15±0.28	293.1±5.86	37.81±3.7	2.38±0.7	0.47±0.06	0.169±0.09	3.1±0.08	44.32±2.6
PT-5A+ 100mM NaCl	24.17±1.46	2.37±0.19	238.1±3.07	42.57±2.6	5.97±0.6	0.39±0.02	0.237±0.12	4.8±0.16	51.08±1.9

Data are given as mean ± standard deviation of three replicates; means followed by same letters are not significant at 5% level

CONCLUSIONS

In comparison to un-inoculated seedlings, our study showed that inoculating wheat seedlings with PGPR isolate PT-5A enhanced their biochemical and physiological state and helped them endure NaCl stress to a greater extent. The PGPR inoculation appears to have a function in reducing the effects of salt stress, as the results show. Thus, PGPR plant inoculation may be a viable solution to reduce NaCl stress-induced damage in wheat seedlings. Nevertheless, additional research must be done to evaluate this strain's effectiveness in alleviating NaCl stress in wheat crops under typical field settings.

FUTURE SCOPE

The results from this study illustrated the positive effect of plant microbe interaction to withstand the wheat seedling under a saline condition. Consequently, PGPR plant inoculation may offer a viable substitute for reducing the harm that saline stress causes to wheat plants. It's possible that the PGPRs employed in this work will be developed into bio-inoculants to protect against salt stress.

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