



## Screening and evaluation of indigenous halo-tolerant microbes for salt stress alleviation in celery (*Apium graveolens*)

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### Abstract

Salinity is one of the major environmental threats which damages growth and productivity of the plants. Microbial assistance in such stressful environment is well recognized. Here in this study, we isolated indigenous microbes and investigated their rescuing potential in celery plants grown under salinity stress. Celery is a widely consumed plant in salads. Plants were cultivated under varying levels (5 & 10% in aqueous solution against control) of salinity in the greenhouse with inocula of two isolated strains of rhizobacteria (RB) which were screened from locally collected soil samples. Data (chlorophylls, carotenoids, anthocyanins, fresh and dry weights of plants, and lengths of root and shoot) were collected and analysed using SPSS. Biochemical isolation of the rhizobacteria was also performed. Plants inoculated with the isolated rhizobacterial strains indicated a statistically significant relief to the stressed plants which resulted in more chlorophylls' (a, b & total), carotenoid and anthocyanin contents that were at par with control. Post inoculation elongation of root and shoot as well as fresh and dry matter accumulations were enhanced significantly. RB 20 indicated statistically significant relief to the plants compared to RB 10. Bacterial strains screening results showed that strains RB 6 & RB 20 proved their positive relieving strengths in the tests of indole synthesis, siderophore production, phosphorus solubilization, casein hydrolysis, catalase activity, citrate biosynthesis, gelatinase biosynthesis, H<sub>2</sub>O<sub>2</sub> production, motility test, osmotic regulation potential and starch hydrolysis. Hence, these indigenous microbes might be helpful in assisting celery plants grown under salinity conditions.

**Keywords:** Salinity, environmental stress, PGPB, celery

### Introduction

Salinity is one of the major environmental concerns that can affect crop productivity and quality and can cause a reduction in the cultivated land area (Shahbaz and Ashraf, 2013). Salt stress is a very important abiotic stress which has affected one third of the world's irrigated land making it unsuitable for cultivation. Plants growth processes like seed germination, seedling development and vigor, flowering, fruit setting etc are adversely affected by salinity stress. Consequently, salinity not only lowers the quality of the produce but also reduces yield (Rabhi *et al.*, 2012). Plants do react to this stress in a number of ways i.e. stopping the entry of salts (at the whole plant or cellular level), taking extra water from soil, decreasing the concentration of the salts in the cytoplasm etc (Teakle and Tyerman, 2010).

Celery is an outstanding verdant vegetable and is specially grown in China, Pakistan, North America, Iran,

Europe and India. Its oil also has helpful applications in pharmaceutical and fragrance industry. The seed of celery has properties like a diuretic, calming and carminative (Marongiu *et al.*, 2013). Celery is a plant with wide uses (Wang *et al.*, 2010).

Salinity has a negative influence on microbial activities going on in the rhizosphere like reduction of microbial growth, community structure change etc (Laderio *et al.*, 2012). However, microbes can tolerate salinity by employing strategies like assembling osmolytes, altering their community structure etc. (Wichern *et al.*, 2006). Soil, plants and microbes strongly interact through soil environment. The non-pathogenic and beneficial rhizospheric microbes referred to as plant growth-promoting bacteria (PGPB) rescue the plants through certain direct and indirect mechanisms (Ramadoss *et al.*, 2013). The nitrogen fixation, phosphate

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solubilization and the production of phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase are direct mechanisms to enhance plant growth. Whereas, indirect mechanisms include production of antagonistic compounds such as hydrolytic enzymes, siderophores and variety of antibiotics (Suárez-Moreno *et al.*, 2012; Costa *et al.*, 2014).

Several studies have highlighted the role of PGPB in the mitigation of salt stress and occurrence of indigenous microbes further provides a strong base camp to better fight the salinity stress (Costa *et al.*, 2014; Lubna *et al.*, 2018). Henceforth, present study was designed to explore the indigenous microbiota which can ease out salinity stress in celery.

## Materials and Methods

### Isolation and inoculum preparation of halo-tolerant bacterial strains

Two most outstanding bacterial strains were isolated at the University of Punjab Lahore from soil samples taken from saline land patches of the Varsity Agricultural Farm randomly. Briefly, Soil was mixed and diluted (8X) with double sterilized water by vertexing. Further 10X dilutions were made and aliquots were shifted onto nutrient agar plates. Purified and dissimilar colonies were further cultured in LB media in flasks which were then centrifuged to obtain the pellets. The pellets were washed with sterilized distilled water and diluted to concentrations of  $10^{7-8}$  cells/mL by taking OD of 1.0 at 600nm. After the isolation of bacterial strains, the isolates were tested for stress alleviation traits mentioned in table 5 and two strains (RB 6 & RB 20) with maximum potential were utilized in further studies (Damodaran *et al.*, 2013). Biochemical isolation data of the rest strains are not shown.

### Application of salinity stress and PGPB to *A. graveolens*

Experiment was performed to see the efficacy of two selected PGPB strains (RB6 and RB20) on growth and development of *A. graveolens* (Celery) in the greenhouse generating salinity by applying defined volumes of solutions of 5% and 10% NaCl salt. The experimental design consisted of nine sets of celery plants with: (1) 5% NaCl solution ( $T_1$ ), (2) 10% NaCl solution ( $T_2$ ), (3) strain RB6 + 0% NaCl solution ( $T_3$ ), (4) strain RB20 + 0% NaCl salt solution ( $T_4$ ), (5) strain RB6 + 5% NaCl salt solution ( $T_5$ ), (6) strain RB20 + 5% NaCl salt solution ( $T_6$ ), (7) strain RB6 + 10% NaCl salt solution ( $T_7$ ), (8) strain RB20 + 10% NaCl salt solution ( $T_8$ ), (9) Control having only distilled water ( $T_9$ ).

Celery seeds were sterilized with sodium hypochlorite (1% for 15 minutes followed by 3 to 4 washings with distilled water). Nursery was raised, and 5 seedlings were sown into each pot containing 1 kg sterilized soil (Table 1) and left for 2 weeks to get established. Plants were supplied with distilled water till establishment. Then, the above-mentioned treatments were applied to the pots randomly using 50mL of each of the treatment solutions (salt solution & aqueous bacterial solution with adjusted concentration). Same amount of distilled sterilized water was given to the control. Plants were kept watering with salt/control solutions on daily basis.

Harvesting of the plants was done at 30 days post inoculation. The plant growth parameters including shoot length, root length, fresh and dry biomasses were recorded. There were total five replicates.

**Table 1: Soil properties**

| Parameters                    | Results            |
|-------------------------------|--------------------|
| pH                            | 7.3 $\pm$ 0.62     |
| ECe ( $\mu\text{S cm}^{-3}$ ) | 404 $\pm$ 26.09    |
| P ( $\text{mg kg}^{-1}$ )     | 193.7 $\pm$ 110.55 |
| Organic Matter                | 1.20 $\pm$ 0.27    |
| Nitrogen (%)                  | 0.016 $\pm$ 0.05   |
| Sand (%)                      | 59.3 $\pm$ 4.7     |
| Silt (%)                      | 31.2 $\pm$ 2.1     |
| Clay (%)                      | 9.3 $\pm$ 0.78     |
| Texture                       | Sandy loam         |

### Estimation of chlorophylls, anthocyanin and total carotenoids

The estimations of Chlorophyll 'a', 'b', total chlorophyll, anthocyanin and carotenoid were done by using the methods as described by Arnon (1949) and Lichtenthaler and Wellburn (1983), respectively.

### Biochemical characterization of bacterial strains used for plant growth promotion

Auxins (indole acetic acid/IAA) production (Benizriet *et al.*, 1998), siderophore production (Perez-Miranda *et al.*, 2007), phosphate solubilisation (Pikovskaya, 1948), casein hydrolysis (Prescott, 2002), catalase activity and citrate consumption (Prescott, 2002), gelatinase liquefaction (Blazevic and Ederer, 1975), hydrogen sulphide synthesis (Prescott, 2002), osmotic pressure (Geetha *et al.*, 2014) and starch hydrolysis (Prescott, 2002) tests were also performed to mark the bacterial potency.

### Data analysis

Data obtained were analysed using ANOVA with the



help of SPSS (V. 10) and means were separated by using Duncan's multiple range test at 5% level.

## Results

### Screening of bacterial strains for plant growth promoting traits

#### Impact of PGPB strains on growth parameters of plants

A significant impact of both the bacterial strains was seen on growth and vigour of *A. graveolens* plants. An increase of shoot length by 13% and 21% was recorded after the inoculation of strains RB6 and RB20, respectively, under 10% salinity stress conditions compared with control.

salinity stress), accordingly (Table 2). Similarly, fresh and dry biomasses of the plants were significantly higher over the introduction of RB20 under low level of salinity stress (5%) compared with both the control and RB6, however, both the bacterial isolates were statistically at par at 10% salinity conditions (Table 3).

#### Impact of PGPB strains on chlorophylls, carotenoids and anthocyanin contents of the plants

Photosynthetic apparatus (chlorophyll *a*, chlorophyll *b* and total chlorophyll), carotenoids and anthocyanin production were enhanced upon furnishing of PGPB as compared to control in both the salinity stress levels.

**Table 2: Influence of PGPB strains on shoot and root lengths of the plants at various salt stresses.**

| Treatment | Control (0% salt)     |                        | Salinity level 1 (5% salt) |                       | Salinity Level 2 (10% salt) |                       |
|-----------|-----------------------|------------------------|----------------------------|-----------------------|-----------------------------|-----------------------|
|           | Shoot Length          | Root length            | Shoot Length               | Root length           | Shoot Length                | Root length           |
| Water     | 7.2±0.47 <sup>c</sup> | 4.6±0.21 <sup>c</sup>  | 6.7±0.33 <sup>c</sup>      | 4.7±0.13 <sup>c</sup> | 7.1±0.68 <sup>c</sup>       | 4.2±0.28 <sup>c</sup> |
| RB6       | 9.8±0.93 <sup>a</sup> | 6.7±0.43 <sup>b</sup>  | 7.3±0.50 <sup>b</sup>      | 4.9±0.49 <sup>b</sup> | 8.2±0.39 <sup>b</sup>       | 5.7±0.13 <sup>b</sup> |
| RB20      | 8.1±0.52 <sup>b</sup> | 7.11±0.57 <sup>a</sup> | 7.8±0.72 <sup>a</sup>      | 5.3±0.69 <sup>a</sup> | 9.1±0.93 <sup>a</sup>       | 6.8±0.41 <sup>a</sup> |

Five independent replicates were averaged into presented values. Values given with ± sign refers to standard errors. Alphabetical letters indicate significance level obtained by applying ANOVA and DMRT ( $p=0.05$ ). Data were gathered at 30th post inoculation day.

**Table 3: Influence of PGPB strains on fresh and dry biomasses of the plants at various salt stresses.**

| Treatment | Control (0% salt)      |                          | Salinity level (5% salt) |                         | Salinity Level 2 (10% salt) |                          |
|-----------|------------------------|--------------------------|--------------------------|-------------------------|-----------------------------|--------------------------|
|           | Fresh Weight           | Dry Weight               | Fresh Weight             | Dry Weight              | Fresh Weight                | Dry Weight               |
| Water     | 2.99±0.48 <sup>c</sup> | 0.196±0.039 <sup>c</sup> | 0.19±0.21 <sup>c</sup>   | 0.16±0.02 <sup>b</sup>  | 0.16±0.63 <sup>a</sup>      | 0.023±0.006 <sup>c</sup> |
| RB6       | 3.76±0.46 <sup>b</sup> | 0.357±0.068 <sup>b</sup> | 0.23±0.38 <sup>b</sup>   | 0.22±0.06 <sup>ab</sup> | 0.20±0.34 <sup>a</sup>      | 0.030±0.001 <sup>b</sup> |
| RB20      | 4.48±0.74 <sup>a</sup> | 0.429±0.047 <sup>a</sup> | 0.33±0.43 <sup>a</sup>   | 0.29±0.07 <sup>a</sup>  | 0.28±0.19 <sup>a</sup>      | 0.040±0.004 <sup>a</sup> |

Five independent replicates were averaged into presented values. Values given with ± sign refers to standard errors. Alphabetical letters indicate significance level obtained by applying ANOVA and DMRT ( $p=0.05$ ). Data were gathered at 30th post inoculation day.

**Table 4: Impact of PGPB strains on chlorophyll 'a' & 'b', total chlorophylls, carotenoids and anthocyanin contents of *A. graveolens* under different treatments**

| Treatment     |                 | Chlorophyll<br>'a' (µg g <sup>-1</sup> ) | Chlorophyll<br>'b' (µg g <sup>-1</sup> ) | Total Chlorophyll<br>(µg g <sup>-1</sup> ) | Carotenoids<br>(µg g <sup>-1</sup> ) | Anthocyanin<br>(µmol g <sup>-1</sup> ) |
|---------------|-----------------|------------------------------------------|------------------------------------------|--------------------------------------------|--------------------------------------|----------------------------------------|
| Blank         | Control         | 4.713±0.37 <sup>bc</sup>                 | 7.127±0.78 <sup>e</sup>                  | 11.84±1.14 <sup>c</sup>                    | 5.096±0.50 <sup>e</sup>              | 0.061±0.004 <sup>d</sup>               |
|               | RB6             | 5.053±0.74 <sup>b</sup>                  | 7.329±0.71 <sup>d</sup>                  | 12.382±0.32 <sup>b</sup>                   | 7.838±0.88 <sup>a</sup>              | 0.097±0.008 <sup>b</sup>               |
|               | RB20            | 5.805±0.81 <sup>a</sup>                  | 8.537±0.79 <sup>a</sup>                  | 14.342±1.41 <sup>a</sup>                   | 7.279±0.89 <sup>b</sup>              | 0.120±0.98 <sup>a</sup>                |
| 5%<br>Saline  | Only salinity   | 1.595±0.22 <sup>f</sup>                  | 5.476±0.47 <sup>h</sup>                  | 7.071±0.79 <sup>h</sup>                    | 4.872±0.13 <sup>f</sup>              | 0.042±0.006 <sup>e</sup>               |
|               | Salinity + RB6  | 4.000±0.38 <sup>d</sup>                  | 5.637±0.53 <sup>g</sup>                  | 9.637±0.99 <sup>g</sup>                    | 5.605±0.45 <sup>d</sup>              | 0.061±0.005 <sup>d</sup>               |
|               | Salinity + RB20 | 4.166±0.41 <sup>cd</sup>                 | 7.087±0.74 <sup>d</sup>                  | 11.253±1.21 <sup>d</sup>                   | 6.029±0.57 <sup>c</sup>              | 0.074±0.006 <sup>c</sup>               |
| 10%<br>Saline | Only salinity   | 0.904±0.08 <sup>i</sup>                  | 5.395±0.46 <sup>i</sup>                  | 6.299±0.58 <sup>i</sup>                    | 1.149±0.11 <sup>i</sup>              | 0.028±0.002 <sup>f</sup>               |
|               | Salinity + RB6  | 3.084±0.29 <sup>e</sup>                  | 6.926±0.69 <sup>f</sup>                  | 10.01±0.99 <sup>f</sup>                    | 4.537±0.51 <sup>h</sup>              | 0.054±0.004 <sup>d</sup>               |
|               | Salinity + RB20 | 3.625±0.27 <sup>de</sup>                 | 7.006±0.75 <sup>e</sup>                  | 10.631±1.10 <sup>e</sup>                   | 4.737±0.49 <sup>g</sup>              | 0.060±0.005 <sup>d</sup>               |

Five independent replicates were averaged into presented values. Values given with ± sign refers to standard errors. Alphabetical letters indicate significance level obtained by applying ANOVA and DMRT ( $p=0.05$ ). Data were gathered at 30th post inoculation day.

Likewise, 26% and 38% increase in root length was noticed post inoculation of microbial isolates RB6 and RB20 in comparison with control under higher salinity level (10%

Further, strain RB20 showed significantly better results than RB6 (Table 4). When compared at 5% salinity level, the percent increases in chlorophyll 'a,' chlorophyll 'b,' total



chlorophyll, carotenoids and anthocyanin syntheses contributed by strain RB20 were 62, 23, 37, 19 and 43%, accordingly, compared with the plants grown without inoculum (Table 4). The assistance provided by the microbial isolates of both the types (RB6 & RB20) in achieving the higher syntheses of chlorophylls, carotenoids and anthocyanin was also statistically comparable to the control plants grown with distilled water applications at 5% salinity level (Table 4). As a whole, the assistance provided by strain RB20 in synthesis and maintenance of chlorophylls, carotenoids and anthocyanin was significantly higher than strain RB6 (Table 4).

### Biochemical traits screening of the PGPB strains

A comparative depiction of the overall scenario of the biochemical traits of the strains is given in Table 5. Results exhibited that strain RB20 performed significantly better than strain RB6 in IAA synthesis, siderophore production than strain RB6 whereas RB6 failed to show phosphate solubilisation contrary to RB 20 (Table 5). For citrate

### Discussion

PGPB have been reported in relieving plants under salinity stress by improving the overall growth performance of the plants (Damodaran *et al.*, 2013; Lubna *et al.*, 2018). Plant roots explore the soil and provide nutrients and other benefits to the plants. Hence, any impediment in root expansion also disturbs rest of the plant functions including shoot growth. But the presence of plant growth promoting bacteria in the rhizosphere can alleviate this stress and enable the plant to continue its normal functioning (Tank and Saraf, 2010; Ramadoss *et al.*, 2013; Lubna *et al.*, 2018). Our results also confirm that the presence of excessive amount of salts in the soil not only affects the root system elongation but also shoot growth of the celery plants. However, induction of the microbial isolates benefited the plants and restored their overall growth to a larger extent (Table 2). Similarly, salinity adversely affects the photosynthetic pigments of the plants which in turn make plant growth and development stunted (Rabhi *et al.*, 2012; Sang-Mo *et al.*, 2014). Application of PGPB improves the integrity and biosynthesis of chlorophylls (Tank and Saraf,

**Table 5: Biochemical trait screening of the PGPR strains**

| Biochemical Trait              | Strain 1 (RB6) | Strain 2 (RB20) |
|--------------------------------|----------------|-----------------|
| IAA production                 | +              | ++              |
| Siderophore synthesis          | +              | ++              |
| Phosphorus solubilization      | -              | +               |
| Casein hydrolysis              | +++            | +++             |
| Catalase activity              | -              | -               |
| Citrate consumption            | +++            | ++              |
| Gelatinase liquefaction        | -              | -               |
| Hydrogen sulphide production   | +++            | +++             |
| Motility presence              | ++             | +               |
| <b>Osmotic regulation test</b> |                |                 |
| Control                        | +++            | +++             |
| 5% salinity                    | +++            | +++             |
| 10% salinity                   | ++             | ++              |
| Starch hydrolysis              | +++            | -               |

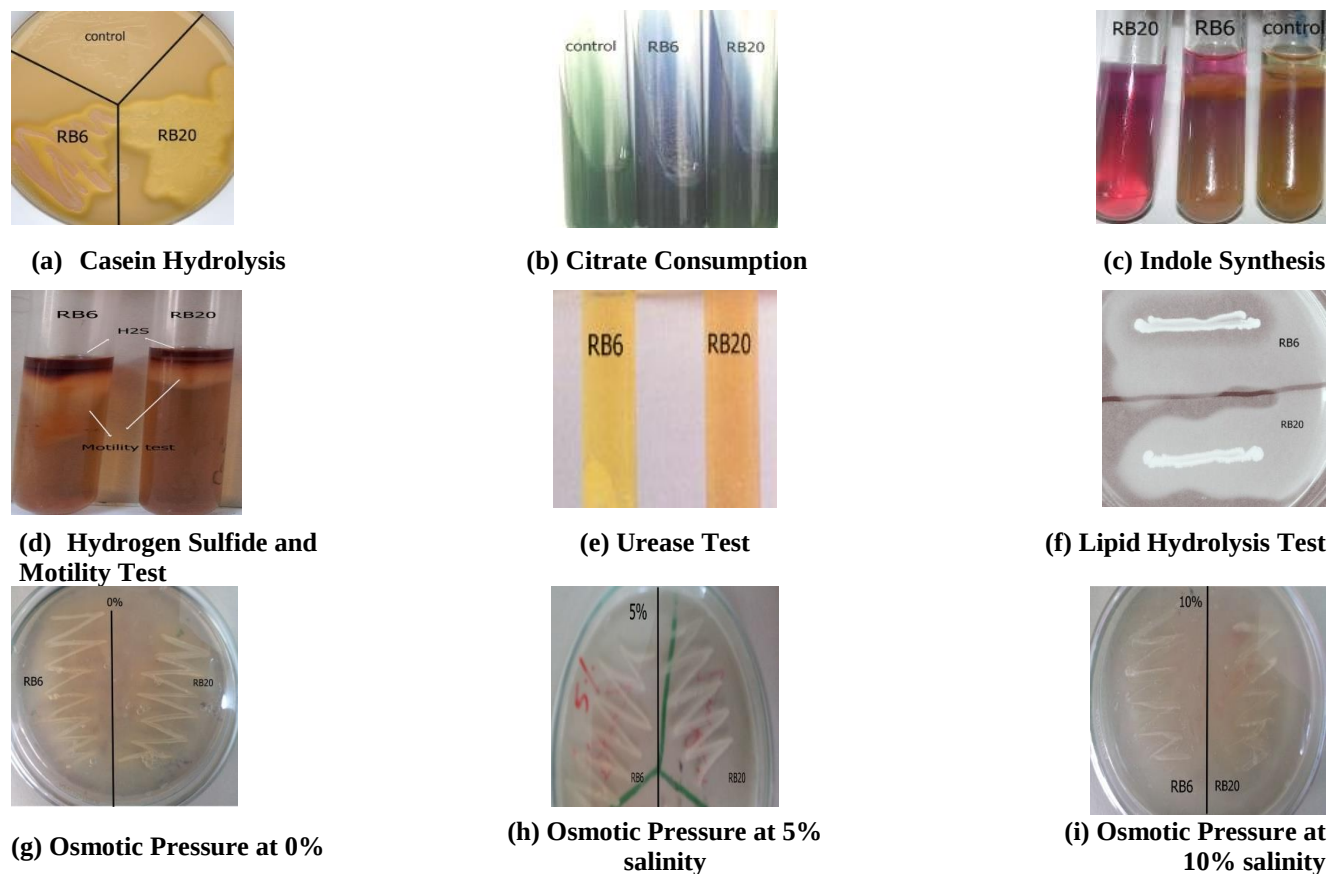
Note; (-) = no synthesis, (+) = low synthesis, (++) = intermediate synthesis, (+++) = high synthesis.

utilization test, both the strains showed positive influence, however, RB6 expressed a significantly better result as compared to strain RB20 (Table 5). Motility test confirmed the positive involvement of both the strains but RB6 was found superior to strain RB20 (Table 5). Biosynthesis of indole acetic acid was less significant in RB6 than in RB20. Osmotic contents regulation by both the strains was statistically equal except for hydrolysis of starch where a negative result was obtained under RB20 strain application and it was lowered under higher salinity level in both of the strains' applications (Table 5). Similar confirmations could also be seen in Figure 1.

2010; Sang-Mo *et al.*, 2014). Table 4 clearly indicates the enhanced synthesis of chlorophylls, carotenoids and anthocyanins after the inoculations were provided to the experimental pots. Upregulated biosynthesis of photosynthetic apparatus lead to increased biomass accumulation by celery plants in our study.

Above mentioned relief to the plants is an outcome of the ability of the microbes to support higher productions of the biochemicals involved in rescue activities (Table 5). Better performance by plants under RB20 strain application was probably due to its higher ability to support various





**Figure 1: Biochemical screening of PGPB strains**

chemicals/functions which helped the plants to fetch more benefits to the plants than RB6 (Table 5). Improved osmotic regulation under salinity stress was noticed upon introduction of isolates to the pots in our experiment (Figure 1 & Table 5). Indole acetic acid, siderophore release, phosphorus solubilization, casein hydrolysis, catalase activity, citrate consumption, gelatinase liquefaction, hydrogen sulphide production, osmotic regulation all are the biochemical/traits associated with relief functions in plants under stressful salinity environments (Nadeem *et al.*, 2012; Ribeiro and Cardoso, 2012; Suárez-Moreno *et al.*, 2012; Damodaran *et al.*, 2013; Geetha *et al.*, 2014; Lubna *et al.*, 2018).

## Conclusion

A significant enhancement of growth parameters of plants like shoot length, root length, plant fresh biomass and dry biomass upon treatment with PGPB alone in comparison with control. A similar boost was seen when PGPB were applied under salinity conditions. All this was achieved as a result of relief provided to the plants by the microbial

inoculations applied. Hence, indigenous microbiota may also be employed in enhancing growth of celery plants under saline conditions.

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