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# Large Scale Multiplication of *Casuarina junghuhniana* Miq. Clonal Plants through Mini-cutting Technique

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**Abstract:** The modern concept of meeting the customer's requirements in better products at low costs in a sustainable manner is possible only through innovative methods. The nodal cutting technique is the most widely used method for large scale propagation of *Casuarina*, *Eucalyptus* and other pulpwood species in India. Tamil Nadu Newsprint and Papers Limited (TNPL) has started large scale multiplication of *Casuarina junghuhniana* Miq. using mini-cutting technique from indoor clonal mini hedges raised in sand beds. When compared to stem/nodal cuttings, indoor clonal mini hedges raised in sand beds improve the rooting potential, quality of root systems and are time- and cost-saving. The productivity of cuttings is increased five times in indoor clonal hedge orchard than conventional stem/nodal cutting. The rooting percentage also improved to 90% without rooting hormone whereas the same is only 50% in stem cutting. The plant developed through mini-cutting technique has more lateral root system which helps the plants/trees to withstand during heavy winds. Replacing such stump derived stock plants by intensively managing indoor sand bed clonal mini hedges resulted in a noticeable enhancement of cutting capacity for adventitious rooting as well as the overall quality of the plants produced in much shorter period with easier and cheaper maintenance. The study reveals that mini-cutting method is ideal propagation technique for large scale propagation of *C. junghuhniana* clones in India.

**Key words:** *Casuarina junghuhniana*, mini-cutting, stem cutting, sand bed and propagation.

## 1. Introduction

*Casuarina junghuhniana* Miq. is native to the highland region of Eastern Indonesia and distributed in Java, Bali, Lombok, Sumbawa, Flores, Sumba, Timor and the Sunda group of islands [1, 2]. It is a promising fast growing and nitrogen fixing tree. It is locally important in Indonesia for fuel wood, poles and soil conservation. It is wholly tropical in distribution and is a native of highlands in Indonesia where it pioneers deforested lands such as rocky slopes and grasslands, and in disturbed areas it replaces mixed mountain forest plant communities [3]. It also appears well adapted to growing on alkaline soils in Timor. It is considered one of the moderate drought resistant species and especially good as a pioneer on landslide-prone soils [4]. As like other Casuarinas, wood of *C. junghuhniana* is highly suitable for pulp and paper, in addition to being used as fuel wood and raw material for charcoal production. Calorific value in *C. junghuhniana* charcoal form is 7,180 kcal/kg, among the highest in firewood species. The wood is also very heavy, having an air-dry density of 900 kg/m$^3$. The species is also used for live fencing, soil improvement, building material, etc.

*Casuarina* subspecies *junghuhniana* typically grows in extensive pure stands on volcanic slopes between altitudes of 1,500 m to 3,100 m but can also occur below 100 m. Subspecies *timorensis* is normally found at lower altitudes, especially in Timor where it grows from near sea level to 300 m. Rainfall in its natural habitat is monsoonal in the range of 700-1,500 mm with a well-defined summer [3]. It is also found along gravelly stream beds in Timor. Once trees reach a few meters in height they are fire resistant and have good sprouting ability if fire damaged. *C. junghuhniana* grows in a wide range of soils from volcanic, sandy to compact clay including very acidic sites, pH 2.8 [5]. It also appears well adapted to growing on alkaline soils in Timor. It can tolerate water logging up to 104 d. It is considered moderate to

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highly drought resistant [3, 4]. In Timor, it commonly grows on umestone-derived soils.

In the present scenario of climate change and unprecedented changes in rainfall patterns and deterioration & degradation of the existing natural resources, the most important aspect of forestry is afforestation programme. Getting quality planting material is the key factor in successful afforestation programme. Mass vegetative propagation has become an important tool for increasing the productivity of plantation raised by the forest based industries. There is also a need for new improved propagation techniques for production of clonal planting stock which will be useful for various genetic improvement activities like cloning of selected superior phenotypes, establishing clone banks and clonal seed orchard. The feasibility of mass propagation for industrial plantations came with the development of operational cloning systems usually with rooted cuttings and greater awareness of the benefits of clonal technologies [6]. The demand for vegetative propagated planting stock of *C. junghuhniana* has increased due to uniform growth, high yield and good income to the farmers.

Hence, clonal forestry has high potential for increasing productivity of *C. junghuhniana* in Tamil Nadu. Although the practice of rooting stem cuttings has been very important in establishing clonal forestry, it has limitations, such as loss of rooting ability due to ontogenetic aging of the hedges. New technologies involving rooting of micro- or mini-cuttings have improved rooting potential, rooting speed, root system quality, and reduced costs and have shown their potential to substitute for rooted stem cuttings [7]. To overcome the disadvantages in the conventional vegetative propagation in *C. junghuhniana* and for the rapid transfer of genetic gains, it is necessary to standardize the mini-cutting technique for the above species in Indian conditions for large scale production.

## 2. Materials and Methods

The present study was carried out at Clonal Propagation and Research Centre, Tamil Nadu Newsprint and Papers Limited (TNPL), Kagithapuram (11°03′ N, 77°59′ E), Karur, Tamil Nadu, India.

### *2.1 Sand Beds*

TNPL developed sand beds in the dimension of 18 m × 1 m × 0.3 m using the glass fiber reinforced plastics (GFRP) troughs, which is non-corrosive, and easy to handle. Six troughs of each 3 m length had been used to get 18 m length bed. These troughs were placed on the 1.0 m elevated GI angle rails from the ground level. The each trough was having the drainage hole and is interlinked and connected with drain pipeline. The troughs were filled with 10 mm blue metal for 10 cm and remaining 20 cm filled with sterile sand for easy drainage of excess chemicals and water. The sand beds were covered with 200 micron ultraviolet poly film on top.

### *2.2 Planting in Sand Beds*

*C. junghuhniana* clonal ramets of two clones namely TNFD CJ 1 and IFGTB CJ 9 each plant having 20 cm height were selected and treated with 0.2% carbendazim solution. These ramets were planted in the sand beds with a spacing of 10 cm × 10 cm after wetting the entire sand beds. About 1,800 clonal ramets were planted in 18 m length troughs at 100 clonal ramets/$m^2$.

### *2.3 Input Management*

*C. junghuhniana* clonal plants were grown in sterile sand beds with micro- and macro-nutrients, supplied through inline drip laterals on daily basis and weekly basis in addition to watering twice in a day. Different combinations of fertigations were tested and combination of 0.05 g of urea, 0.009 mL of phosphoric acid, 0.0033 g of potassium nitrate, 0.06 g of sulphate of potash, 0.0033 g of complex, 0.022 g of mono ammonium phosphate and 0.013 g of calcium ammonium nitrate, 0.0013 g of zinc sulphate, 0.0013 g of ferrous sulphate and 0.015 mL of humic acid per plant per day were given. In addition, 0.01 mL of panchakavya per plant per week was also given.

*2.4 Cutting Production*

The plants were maintained up to 60 d by applying the nutrients as per schedule and the plants were pruned to produce new sprigs. Pillared apical shoots of 8-10 cm long were harvested after 70 d and sprigs were immediately dipped in 2% carbendazim solution. Similarly conventional stem cuttings were harvested from hedge gardens and maintained in the open field having the plant density of 4,000 plants per acre at the spacing of 1 m × 1 m. The cuttings were prepared by trimming in cutting shed and planted in 90 cc root trainers filled with decomposed coir pith (Fig. 1). IBA 4,000 ppm was used for the cutting collected from open field clonal hedge orchard whereas there was no rooting hormone that was used for the cuttings collected from clonal mini-hedges in sand beds (Fig. 2).

The planted root trainers were placed inside the mist chamber benches, where the temperature regime of 32-35 °C, relative humidity 85%-90% were maintained during the natural day length period (Fig. 3). The watering was done for 45 s at every 30 min interval. The imposed conditions were relaxed on 15th, 25th and 35th ($T_1$, $T_2$ and $T_3$) days after planting to air prune the roots in different mist chambers for *C. junghuhniana* clones under observation. They were transferred to the 50% hardening chamber 7 d after relaxing the conditions and the water duration was changed from 45 s all 30 min to 15 s for all 2 h (Fig. 4).

*2.5 Acclimation of Rooted Cuttings*

After 5-7 d in shade house the young rooted *C. junghuhniana* clone ramets were placed in the open nursery for 25-30 d and watering was done twice per day for 15 min (Fig. 5). The NPK 19-19-19 was applied at the rate of 0.5 g/plant during this hardening period. Carbendazim 2 g/L or Dithane M45 2 g/L and Triazophos 2 mL/L or Metasystox 2 mL/L were applied to protect the clone ramets from insect pest and diseases.

**Fig. 1 *Casuarina junghuhniana* clone ramets trimming process.**

**Fig. 2 *C. junghuhniana* clone ramets taken from sand bed clonal hedges.**

**Fig. 3 *C. junghuhniana* clone ramets planted in mist chamber.**

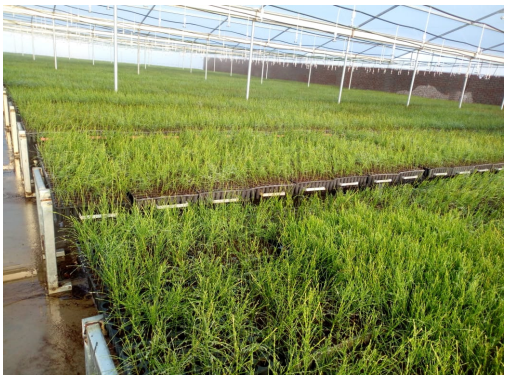

**Fig. 4 *C. junghuhniana* clone ramets rooting in mist chamber.**

**Fig. 5 C. *junghuhniana* clone ramets ready for dispatch in open nursery.**

**Table 1 CJ 1 & CJ 9 mini-cutting and stem cutting growth performance data in mist chamber.**

| Days | 15th day | | 25th day | | 35th day | |
|---|---|---|---|---|---|---|
| Clone | CJ 1 | CJ 9 | CJ 1 | CJ 9 | CJ 1 | CJ 9 |
| (1) Survival (%) | | | | | | |
| Mini cutting | 92.0 ± 1.34$^{b}$ | 97.8 ± 0.83$^{a}$ | 82.0 ± 0.70$^{d}$ | 90.0 ± 1.58$^{b}$ | 80.0 ± 1.58$^{d}$ | 85.0 ± 1.58$^{c}$ |
| Stem cutting | 75.0 ± 0.44$^{b}$ | 80.0 ± 0.54$^{a}$ | 60.0 ± 0.70$^{d}$ | 70.0 ± 0.63$^{c}$ | 50.0 ± 0.70$^{e}$ | 60.0 ± 0.89$^{d}$ |
| (2) Root length (cm) | | | | | | |
| Mini cutting | 2.24 ± 0.06$^{e}$ | 2.50 ± 0.04$^{e}$ | 4.50 ± 0.19$^{d}$ | 5.30 ± 0.28$^{c}$ | 6.80 ± 0.20$^{b}$ | 7.60 ± 0.18$^{a}$ |
| Stem cutting | 1.80 ± 0.12$^{f}$ | 2.30 ± 0.09$^{e}$ | 3.80 ± 0.44$^{d}$ | 5.10 ± 0.16$^{c}$ | 5.60 ± 0.16$^{b}$ | 6.80 ± 0.13$^{a}$ |
| (3) Lateral roots (No.) | | | | | | |
| Mini cutting | 2.00 ± 0.31$^{d}$ | 2.20 ± 0.20$^{d}$ | 3.20 ± 0.37$^{c}$ | 4.20 ± 0.37$^{b}$ | 4.80 ± 0.20$^{b}$ | 5.80 ± 0.20$^{a}$ |
| Stem cutting | 1.60 ± 0.24$^{c}$ | 2.00 ± 0.31$^{c}$ | 2.00 ± 0.31$^{c}$ | 2.40 ± 0.24$^{b}$ | 3.20 ± 0.20$^{ab}$ | 4.00 ± 0.31$^{a}$ |
| (4) Shoot length (cm) | | | | | | |
| Mini cutting | 10.00 ± 0.31$^{e}$ | 12.00 ± 0.54$^{d}$ | 12.60 ± 0.24$^{d}$ | 14.20 ± 0.58$^{c}$ | 17.80 ± 0.37$^{b}$ | 19.20 ± 0.37$^{a}$ |
| Stem cutting | 9.80 ± 0.48$^{d}$ | 10.60 ± 0.50$^{cd}$ | 11.60 ± 0.50$^{c}$ | 14.20 ± 0.58$^{b}$ | 15.20 ± 0.37$^{b}$ | 18.80 ± 0.58$^{a}$ |

Values are means of five replicates recorded after 15th day, 25th day and 35th day.

Values in the last three columns are mean ± SE of mean followed by the letters within the column indicating the level of significance at $p < 0.05$ by Duncan's multiple range test.

*2.6 Statistical Analysis*

The following parameters were assessed to draw the observations in conventional stem cutting method and mini-cutting technique.

(1) The clone ramets survival, shoot length, root length, lateral roots after 15th, 25th and 35th ($T_1$, $T_2$ and $T_3$) days in the mist chamber.

(2) The clone ramets survival, shoot length, root length, lateral roots were measured in hardening chamber 20 d after transfer from 50% mist chamber for $T_1$, 13 d after transfer for $T_2$ and 6 d after transfer for $T_3$.

(3) The clone ramets survival, shoot length, root length, lateral roots were measured in open nursery 20 d after transfer from 50% hardening chamber for $T_1$, 13 d after transfer for $T_2$ and 6 d after transfer for $T_3$.

The experiment was laid in a randomized complete block design and compared by least significant difference (LSD) at 5% probability level.

## 3. Results and Discussion

The results of the conventional stem cutting method and mini-cutting technique for *C. junghuhniana* clonal plants were presented in Tables 1-4. The number of days required for the optimal rooting for two methods revealed that mini-cutting technique survival was 90% whereas conventional cutting method 60% only within the treatment period (Table 5).

Mini-cuttings have the ability to produce more lateral root system. The length of this root system was

**Table 2 CJ 1 & CJ 9 mini-cutting and stem cutting growth performance data in hardening chamber.**

| Days | 6th day | | 13th day | | 20th day | |
|---|---|---|---|---|---|---|
| Clone | CJ 1 | CJ 9 | CJ 1 | CJ 9 | CJ 1 | CJ 9 |
| (1) Survival (%) | | | | | | |
| Mini cutting | 84.0 ± 0.89$^{c}$ | 98.0 ± 0.31$^{a}$ | 72.0 ± 1.30$^{d}$ | 91.0 ± 0.31$^{b}$ | 67.0 ± 0.70$^{e}$ | 90.0 ± 0.31$^{b}$ |
| Stem cutting | 72.0 ± 0.31$^{b}$ | 84.0 ± 0.83$^{a}$ | 60.0 ± 0.44$^{e}$ | 68.0 ± 0.44$^{c}$ | 58.0 ± 0.44$^{f}$ | 62.0 ± 0.44$^{d}$ |
| (2) Root length (cm) | | | | | | |
| Mini cutting | 13.60 ± 0.50$^{c}$ | 14.20 ± 0.37$^{c}$ | 16.20 ± 0.37$^{b}$ | 16.2 ± 0.37$^{b}$ | 17.80 ± 0.37$^{a}$ | 18.20 ± 0.58$^{a}$ |
| Stem cutting | 9.80 ± 0.58$^{e}$ | 12.0 ± 0.31$^{d}$ | 13.20 ± 0.37$^{c}$ | 14.20 ± 0.20$^{b}$ | 14.40 ± 0.50$^{b}$ | 16.40 ± 0.50$^{a}$ |
| (3) Lateral roots (No.) | | | | | | |
| Mini cutting | 3.60 ± 0.24$^{e}$ | 5.20 ± 0.37$^{d}$ | 5.40 ± 0.40$^{d}$ | 6.40 ± 0.40$^{c}$ | 7.40 ± 0.24$^{b}$ | 8.80 ± 0.20$^{a}$ |
| Stem cutting | 2.80 ± 0.37$^{c}$ | 3.00 ± 0.31$^{c}$ | 3.40 ± 0.24$^{c}$ | 5.20 ± 0.37$^{b}$ | 5.80 ± 0.58$^{b}$ | 7.00 ± 0.44$^{a}$ |
| (4) Shoot length (cm) | | | | | | |
| Mini cutting | 16.20 ± 0.37$^{e}$ | 23.20 ± 0.37$^{c}$ | 18.40 ± 0.50$^{d}$ | 24.80 ± 0.37$^{b}$ | 24.60 ± 0.50$^{b}$ | 26.20 ± 0.37$^{a}$ |
| Stem cutting | 15.00 ± 0.70$^{d}$ | 15.20 ± 0.37$^{d}$ | 17.80 ± 0.37$^{c}$ | 20.00 ± 0.44$^{b}$ | 19.60 ± 0.50$^{b}$ | 22.80 ± 0.37$^{a}$ |

Values are means of five replicates recorded after the 6th day, 13th day and 20th day.

Values in the last three columns are mean ± SE of mean followed by the letters within the column indicating the level of significance at $p < 0.05$ by Duncan's multiple range test.

**Table 3 CJ 1 & CJ 9 mini-cutting and stem cutting growth performance data in open nursery.**

| Days | 6th day | | 13th day | | 20th day | |
|---|---|---|---|---|---|---|
| Clone | CJ 1 | CJ 9 | CJ 1 | CJ 9 | CJ 1 | CJ 9 |
| (1) Root length (cm) | | | | | | |
| Mini cutting | 21.20 ± 0.37$^{d}$ | 22.0 ± 0.31$^{cd}$ | 22.80 ± 0.48$^{bc}$ | 23.20 ± 0.37$^{b}$ | 23.60 ± 0.24$^{b}$ | 24.80 ± 0.37$^{a}$ |
| Stem cutting | 17.80 ± 0.37$^{d}$ | 19.80 ± 0.37$^{c}$ | 21.20 ± 0.37$^{b}$ | 22.20 ± 0.37$^{ab}$ | 22.20 ± 0.37$^{ab}$ | 23.20 ± 0.20$^{a}$ |
| (2) Lateral roots (No.) | | | | | | |
| Mini cutting | 6.60 ± 0.24$^{d}$ | 9.80 ± 0.37$^{b}$ | 8.20 ± 0.37$^{c}$ | 10.60 ± 0.50$^{b}$ | 8.60 ± 0.24$^{c}$ | 12.00 ± 0.44$^{a}$ |
| Stem cutting | 5.20 ± 0.37$^{d}$ | 6.00 ± 0.31$^{cd}$ | 6.20 ± 0.37$^{cd}$ | 7.00 ± 0.44$^{bc}$ | 7.80 ± 0.37$^{b}$ | 9.80 ± 0.58$^{a}$ |
| (3) Shoot length (cm) | | | | | | |
| Mini cutting | 24.20 ± 0.37$^{d}$ | 25.40 ± 0.40$^{c}$ | 24.80 ± 0.37$^{cd}$ | 26.80 ± 0.37$^{b}$ | 27.80 ± 0.37$^{b}$ | 29.80 ± 0.37$^{a}$ |
| Stem cutting | 17.60 ± 0.24$^{d}$ | 18.60 ± 0.50$^{d}$ | 20.20 ± 0.83$^{c}$ | 22.20 ± 0.37$^{b}$ | 23.20 ± 0.73$^{b}$ | 27.20 ± 0.48$^{a}$ |

Values are mean of five replicates recorded after the 6th day, 13th day and 20th day.

Values in the last three columns are mean ± SE of mean followed by the letters within the column indicating the level of significance at $p < 0.05$ by Duncan's multiple range test.

**Table 4 CJ 1 & CJ 9 mini-cutting and stem cutting month wise rooting performance.**

| Month | Mini-cutting | Stem cutting |
|---|---|---|
| January | 78.00 ± 0.36$^{e}$ | 40.00 ± 0.93$^{e}$ |
| February | 82.00 ± 0.36$^{d}$ | 44.00 ± 0.36$^{c}$ |
| March | 83.00 ± 0.25$^{cd}$ | 44.00 ± 0.36$^{c}$ |
| April | 82.00 ± 0.68$^{d}$ | 42.00 ± 0.44$^{d}$ |
| May | 85.00 ± 0.36$^{ab}$ | 42.00 ± 0.25$^{d}$ |
| June | 85.00 ± 0.36$^{ab}$ | 46.00 ± 0.36$^{ab}$ |
| July | 86.00 ± 0.25$^{a}$ | 45.00 ± 0.25$^{bc}$ |
| August | 86.00 ± 0.36$^{a}$ | 46.00 ± 0.25$^{ab}$ |
| September | 84.00 ± 0.44$^{bc}$ | 47.00 ± 0.25$^{a}$ |
| October | 83.00 ± 0.36$^{cd}$ | 47.00 ± 0.36$^{a}$ |
| November | 83.00 ± 0.36$^{cd}$ | 46.00 ± 0.36$^{ab}$ |
| December | 82.00 ± 0.57$^{d}$ | 44.00 ± 0.36$^{c}$ |

Values are mean of five replicates recorded after the 35th day.

Values in the last three columns are mean ± SE of mean followed by the letters within the column indicating the level of significance at $p < 0.05$ by Duncan's multiple range test.

**Table 5 Advantages of sand bed mini-cutting technique.**

| Description | Field CMA | Sand bed mini-cutting |
|---|---|---|
| Spacing | 1 m × 1 m | 10 cm × 10 cm |
| Plants/$m^2$ | 1 | 100 |
| 1st harvest | After six months | After one month |
| Harvesting interval | 20 d | 6 d |
| Maintenance | Difficult and need to spend more on keeping the area clean like weeding, watering, fertilizer application, etc. | Easy to maintain and less labour required for fertigation, etc. |
| Shoots production/$m^2$/year | 296 | 4,800 |
| Collection of cuttings | Difficult | Easy |
| Rooting hormone | Required | Not required |
| Rooting initiation | After 20 d | After 13 d |
| Root system | Forming lateral roots in one side due to ununiform irrigation and fertigation which leads to lodging during heavy wind | Forming lateral roots in all sides of the stem like tap root and it helps to withstand during heavy wind due to uniform irrigation and fertigation |
| Rooting percent | 44-52 | 80-90 |
| Nutrient uptake of plants | Poor and influenced by the soil and other environmental factors | Required nutrients absorbed by the plants |

CMA: Clonal Multiplication Area.

measured to find out the number of days required in the mist chamber. The result shows that clones performed well with mini-cutting technique compared to conventional cutting method. The similar results were obtained for shoot length and the number of lateral roots (Table 1). In 50% hardening chamber survival rate is high in mini-cutting method compared to conventional stem/nodal cutting method (Table 2).

The shoot length, root length and the number of lateral roots were measured 20 d after transfer from 50% hardening chamber to open nursery for $T_1$, 13 d for $T_2$ and 6 d for $T_3$. The shoot length was more for the clones from mini-cutting technique compared to conventional stem cutting method in all three treatments. The similar results trend was noticed in root length and no of lateral roots (Table 2). In open nursery the shoot length was more in clones from mini-cutting technique compared to conventional stem cutting method in all three treatments. The similar results trend was noticed in root length and the number of lateral roots (Table 3).

The results of the present study show the significant improvements in all the stages of propagation in the case of mini-clonal cutting technique over the conventional clone ramets production through the stem cuttings (Table 4). Rooting response was higher in the mini-cutting compared to the stem cuttings indicating the influence of juvenile cutting [8]. The use of growth substances was completely eliminated in the mini-cutting technique.

The clone ramets, when produced through conventional stem cutting method, are 50% rooting only whereas the same is more than 90% in mini-cutting method (Table 2). The similar results were observed in pulpwood species like *Eucalyptus* and *Casuarina* species [9-17].

The nutritional status of the mother plants highly influence the rooting capability of mini-cuttings. The nutrient schedule for the present study was standardized through the rigorous testing of many nutrient combinations for *C. junghuhniana* clones. The maintenance of juvenile stage also influences the rooting in the mini-cuttings. The same trend was observed in *Eucalyptus* species [12]. The ability of mini- and micro-cuttings to produce superior clones than the stem cuttings was documented in *Eucalyptus* and *Casuarina* species [9-17].

## 4. Conclusions

The present study reveals the suitability of mini-cutting clonal production method for *C.*

*junghuhniana* under Indian conditions. Apart from this the mini-cutting method of clone production completely eliminates the use of growth substances. Clone production through the mini-cutting method reduces the time period in the mist chamber because of its rooting ability in shorter periods. This will ultimately influence the increase in usage of mist chamber. Thus the unit area production rate in mist chamber was increased through the mini-cutting method. The plant developed through mini-cutting technique has more lateral root system which helps the plants/trees to withstand during heavy winds.

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