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**Capacitance Characteristics of *Pinus densata*, *Pinus tabuliformis*, *Pinus yunnanensis* and the hybrids *Pinus tabuliformis* × *Pinus Yunnanensis***

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ARTICLE INFO

*Article history*

Received: 21 September 2020

Accepted: 12 October 2020

Published Online: 31 October 2020

*Keywords:*

*Pinus densata*

*Pinus yunnanensis*

*Pinus tabuliformis*

Hybrid

Kinship

Capacitance

ABSTRACT

We employed capacitance to evaluate the kinship and interspecific variation of homoploid hybrid conifer *Pinus densata*, *P. tabuliformis*, *P. yunnanensis* and artificial hybrids of *P. tabuliformis* (maternal parent) and *P. yunnanensis* (paternal parent) which were cultivated and selected in the common garden experiment. By measuring capacitance spectra under different voltage frequencies, we could differentiate different germplasms based on the electrical response. We aims to demonstrate that *P. densata* as the hybrid of *P. tabuliformis* and *P. yunnanensis* based on the capacitance values of the species, and to provide new evidence to the previously known biological evidence, as well as and the parental effect on the hybrids. Our results revealed that capacitance values between the species are significantly different in the spectra where *P. yunnanensis* positioned at the lowest and *P. densata* was much higher than all other species, indicating that *P. densata* had possessed a great capacity to store electrical energy. The capacitance spectra of *P. densata* and the artificial hybrid are not similar, which rejected our hypothesis. Both of the capacitance values of *P. densata* and the hybrids were closer to *P. tabuliformis* than to *P. yunnanensis*, which shows that the maternal influence was stronger than the paternal influence. Correlation analysis on the relationship between capacitance and fitness-related characteristics showed that capacitance is negatively correlated to mortality rate, and positively correlated with second-year survival rate. High capacitance values of *P. densata* and some of the hybrids reveal their superior adaptability to harsh environment in the Tibet Plateau. We concluded that capacitance as a new indicator for plant fitness and evolution evidence of homoploid hybrid conifers.

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## 1. Introduction

Electrical properties such as resistance, electrical impedance and capacitance are traditionally important tools in medical pathology <sup>[1]</sup>. Recently, these electrical measurements have been used to study the structure of various organic and inorganic materials <sup>[2-4]</sup>, seedling resistance to temperature <sup>[5-6]</sup>, and water content of plant tissues <sup>[7-8]</sup>. In identifying kinship between species and seeking evolutionary evidence, molecular biological method is a fundamental one; however, it is usually complicated and time-consuming regarding lab analysis. Developing a convenient and rapid physical method that can be applied in the field to complement biological methods would be helpful to assist scientists study species relationships in the field.

Capacitance is a basic electrical property that describes the charge storage capability of the conductor system. It is defined as the ratio of the total charge on one conductor to voltage <sup>[9]</sup>. A capacitor can store energy but also consume part of the energy dependent on the applied voltage. Therefore, the capacitor model is a parallel connection of both capacitor and resistor elements. In theory, there is an electric field between conductors of unequal charge potentials, leading to accumulation of charges, electric field energy, and the capacitance effect <sup>[10-11]</sup>. A plant sample can also be considered as a parallel connection of a capacitor element and a resistor element due to its tissue materials and structures.

After applying alternating current (AC) to a plant tissue, the same amount of opposite charges gather at both ends of the sample, and electric field energy then establishes in the sample. After electricity is removed, if the charge continues to stay in the sample, the sample is considered to be capable of storing charges or electric field energy. Therefore, measuring capacitance and evaluating the electrical storage capacity could potentially reveal the inner properties of plant tissues.

Previous molecular biological studies had indicated that *Pinus densata* is a hybrid species originated from *P. tabuliformis* and *P. yunnanensis* <sup>[12]</sup>. In this study, we intended to use capacitance as a complementary method to study such kinship between the three species. We hypothesized that the artificial hybrids of *P. tabuliformis* and *P. yunnanensis* and *P. densata* would have very similar capacitance values. By treating the seedlings of *P. densata*, *P. yunnanensis*, *P. tabuliformis*, and artificial hybrids of *P. densata* and *P. yunnanensis* with AC field treatment, we aimed to detect the differences of their capacitance on a spectral level by applying AC under a range of frequencies and conduct correlation analysis

between capacitance and growth adaptability index to understand the genetic variation within the same species and interspecific kinship, providing the first example for using capacitance as an empirical tool in studying plant kinship and parental effects.

## 2. Materials and Methods

### 2.1 Materials

The artificial hybrids of *Pinus tabuliformis* and *P. yunnanensis* were cultivated in a seed orchard in Ningcheng, Heilihe, Inner Mongolia. The maternal parents were six *P. tabuliformis* clone, and pollen of the paternal parent *P. yunnanensis* was collected from five *P. yunnanensis* in Yunnan, Kunming Province. Thirty hybrids were obtained, including 12 hybrids meeting the basic requirements of field trials. The 12 hybrids were marked as No. 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, and 413). To compare variation between the hybrids and the other species, open-pollinated seeds from the six female clones were used as *P. tabuliformis* samples and mixed seeds from the five pollen trees were used as *P. yunnanensis* samples. Mixed seeds from *P. densata* populations were used as *P. densata* samples. The *P. densata* samples were collected in Linzhi, Tibet, a natural distribution area where no other coniferous species were distributed.

### 2.2 Site Description and Field Experiment

The study site is located in a nursery of Department of Resources and Environment, Tibet Animal Husbandry College in Town of Bayi, Linzhi County (93°25'E, 29°50'N, 2900 m). The area features a plateau-humid monsoon climate zone, with an annual minimum temperature of -5.3 °C, maximum temperature of 22.1 °C, and annual average temperature of 8.5 °C. The annual precipitation is 654.1 mm concentrated between April and October; average annual sunshine hours is 2022.2 hours, and frost-free period is 180 d or more.

Plants were cultivated by seeding beds with the length of 5 m (east-west), width of 1.1 m, and height of 10 cm. The experimental design follows a randomized complete block design with four replications. Each block consists of 12 plots each housing one of the 12 artificial hybrids and the three plots that house the three species. There are 10 plants in each hybrid family plot and 20 plants in each individual species plot. The field planting spacing and management conditions were consistent among all plots.

### 2.3 Measurement of Capacitance

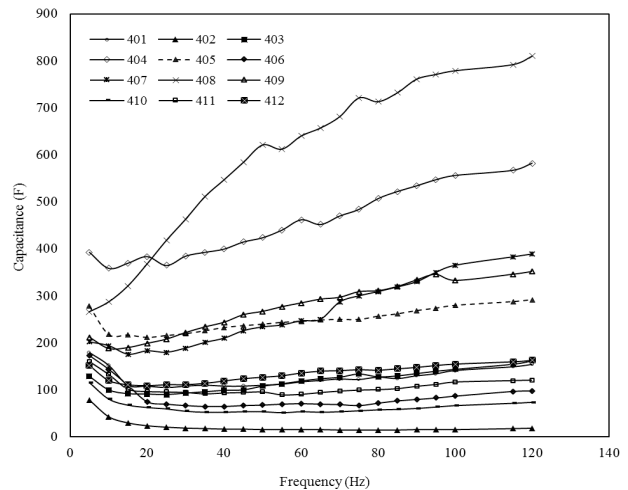
The special electrodes were connected to an impedance

analyzer (TH2828S) made by China Electronics Co. in Changzhou, Jiangsu Province. An open/short circuit correction was conducted to the impedance analyzer prior to measurement. After the adjustment, two seedlings were selected from the artificial hybrids, *P. tabuliformis*, *P. yunnanensis* and *P. densata*, and the measurement was conducted on needle leaves of each seedling without removing them from the seedlings. Each leaf was applied with a voltage of 10 mV, and capacitance values were measured at 53 frequencies between 1 and 100M Hz to develop capacitance spectra. We found that between 1-120 Hz, the spectra curve is more volatile than those at other frequencies, indicating physiological changes in plant cells. Therefore, we selected 1-120 Hz as the measurement range. We randomly sampled healthy needles of *P. tabuliformis*, *P. yunnanensis*, *P. densata* and the hybrid species to measure their capacitance. During the measurement, we avoided physical damage to samples and ensured the stability of the measured results by a non-destructive sampling method. To eliminate the influence of changing atmospheric temperature and light over time, every plant was measured in the morning, at noon and in the afternoon, and the average of three measurements was taken as the final measurement value.

### 3. Results

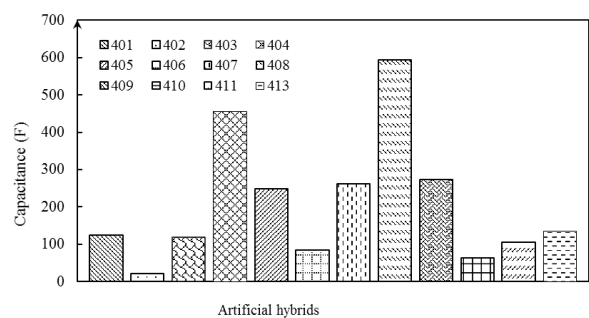
#### 3.1 Capacitance Spectra of the Interspecific Hybrids

With increasing frequency, the capacitance of most hybrids gradually increased, and the overall change was small (except for No. 408) without any large fluctuation (Figure 1). When the voltage frequency was at 20 Hz, the capacitance gap between various hybrids is very clear (Figure 1), indicating the variation in physiological properties of different genotypes of the hybrids. The capacitance of the hybrids tended to slightly decrease between the low frequency span (5-10 Hz) and increased gradually when the frequency increases from 10 to 120 Hz. Notably, the spectra of No.408 and 404 positioned significantly higher than the rest, and the maximum capacitance values are 800 and 500, respectively (Figure1). The rest of hybrids do not have significant change and the gaps between each sample remain relatively constant. The two genotypes have stronger energy storage and discharge capacity for electric field than the other hybrids.

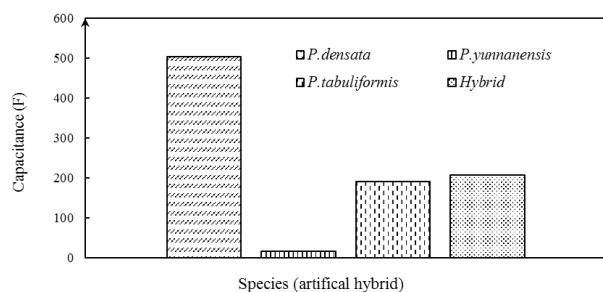


**Figure 1.** Capacitance of 12 hybrids with changing frequencies

In order to more directly compare the variation of capacitance among artificial hybrids and the other three species in the following analysis, we added all the capacitance values of each sample and calculated the average value for the four types of species. Among the hybrids, No.408 and 404 clearly showed significantly higher capacitance than the others (Figure 2a). Among the four species, *P. densata* has the highest capacitance, followed by the hybrid and *P. tabuliformis*, and the capacitance of *P. yunnanensis* is less than 100.



**Figure 2a**

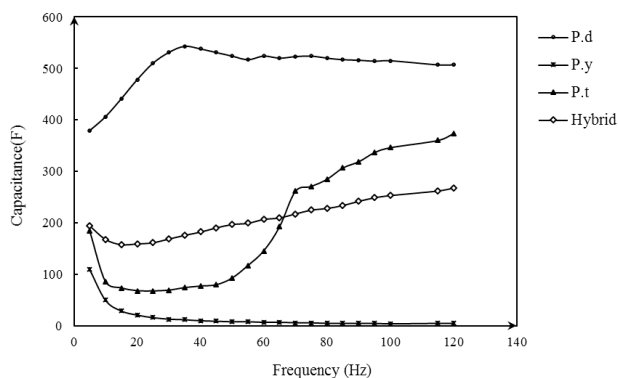


**Figure 2b**

**Figure 2.** (a) Average capacitance values of 12 hybrids across frequencies; (b) Capacitance of *Pinus densata*, *P. tabuliformis* and *P. yunnanensis* and the artificial hybrid

### 3.2 Capacitance Spectra of Artificial Hybrids and the Three Species

Capacitance (average value of all samples) of the hybrids linearly decreased as voltage frequency increased from 5 to 10 Hz and slowly increased with the increasing voltage frequency afterwards (Figure 3). Capacitances of *P. densata* were relatively high and increased linearly when the voltage frequency increased from 5 to 35 Hz, and the capacitance decreased gradually as the voltage frequency increased afterwards, indicating that 35 Hz was the saturation point for *P. densata*. The capacitances of the hybrids were higher than the parental species *P. tabuliformis* and *P. yunnanensis*. With voltage frequency increasing from 80 to 120 Hz, the capacitance of *P. yunnanensis* decreased continuously, indicating that the storage capacity of *P. yunnanensis* was the smallest of all. The capacitance spectra of *P. tabuliformis* feature a spoon-shaped curve. When the voltage frequency was between 5 and 20 Hz, the capacitance slowly decreased, and at 20 Hz it reached the lowest point then rose quickly between frequencies of 70 and 120 Hz. The *P. yunnanensis* has the lowest electrical storage capacity, and storage capacity of the hybrid species was higher than their parental species when the voltage frequency is between 5 and 65 Hz. The capacitances of the hybrids reached the maximum at the frequency of 65 Hz, showing a superior storage capacity than its parental species *P. tabuliformis* and *P. yunnanensis*. After 65 Hz, storage and discharge capacity of the hybrids was larger than *P. yunnanensis* but smaller than *P. tabuliformis*. However, among all the species, the storage capacity of *P. densata* was much higher than that of the hybrids and their parental species. Among the four spectra, the hybrids and *P. densata* were closed to *P. tabuliformis*, showing that maternal influence was stronger than the paternal influence. However, the spectra of the hybrids and *P. densata* are not close to each other.



**Figure 3.** Capacitance spectra of *Pinus densata*, *P. tabuliformis* and *P. yunnanensis* and the hybrid

### 3.3 Modelling of Capacitance and Cluster Analysis of Capacitance

The capacitance spectra of *P. densata*, *P. tabuliformis*, *P. yunnanensis* and the artificial hybrids were modeled by the following equation.

$$C = af^3 + bf^2 + cf + d$$

where  $C$  is the capacitance of a sample,  $a$ ,  $b$ ,  $c$ , and  $d$  are species related parameters, and  $f$  is voltage frequency.

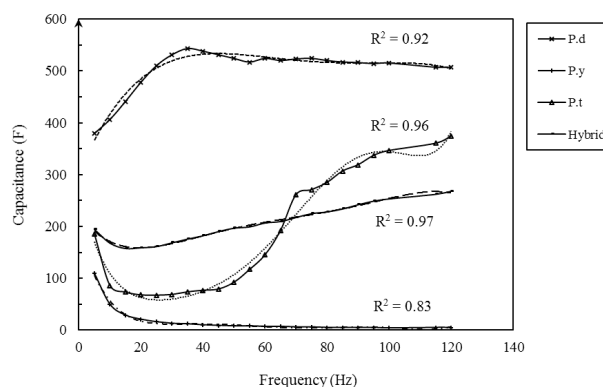
The capacitance varies with the frequency, and *P. yunnanensis* and the artificial hybrids had the best-fit with  $R^2$  value close to 1. The  $R^2$  value for *P. densata* and *P. tabuliformis* were 0.92, 0.83, respectively (Figure 4).

In order to more precisely present the data and simplify the model, each spectrum was segmented and individually modelled. For *P. densata* and *P. tabuliformis*,  $R^2$  value was close to 1 (Figure 5a, b, c and d). *Pinus yunnanensis* was also modelled by segments. Segmented modeling for *P. densata* can be described as follow:

$$C_{p,d} = -1322f^2 + 10812f + 31836 \quad (5 \text{ Hz} \leq f \leq 55 \text{ Hz}), \quad R^2 = 0.99$$

$$C_{p,d} = -0.0022f^2 + 0.1068f + 525.3 \quad (60 \text{ Hz} \leq f \leq 120 \text{ Hz}) \quad R^2 = 0.92$$

Cluster analysis using arithmetic averages were conducted for *P. tabuliformis*, *P. yunnanensis*, *P. densata* and artificial hybrids by grouping the samples based on a capacitance level of 0.4. The samples were clustered into five categories including one hybrid (No. 404) and *P. densata* as the first group, six hybrids (No.413, 403, 401, 411, 402, 410) and *P. yunnanensis* as the second group, three hybrids (No. 405, 407 and 409) and *P. tabuliformis* were clustered into the third group, and two additional hybrids (No. 408 and 406) were the other two groups.



**Figure 4.** Capacitance modelling of *Pinus densata* (P.d), *P. tabuliformis* (P.t) and *P. yunnanensis* (P.y) and the hybrid of *P. tabuliformis* and *P. yunnanensis* (Hybrid)

Cluster analysis showed that the capacitance can clearly distinguish the species (Figure 6). It showed that the

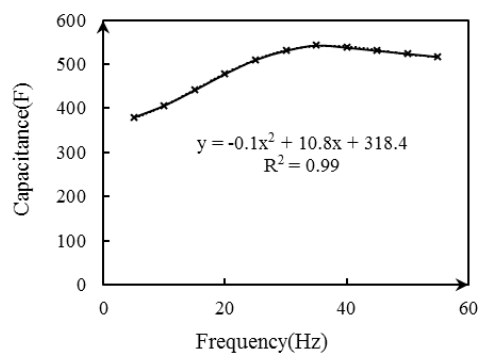


Figure 5a

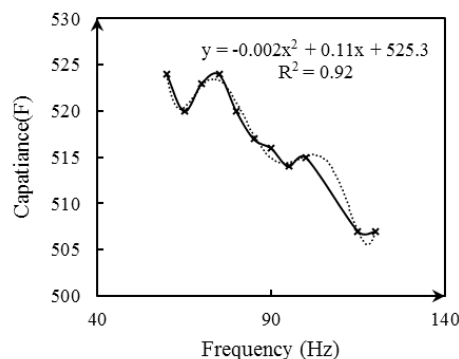


Figure 5b

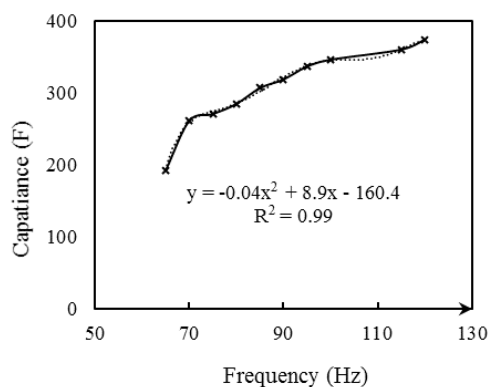


Figure 5c

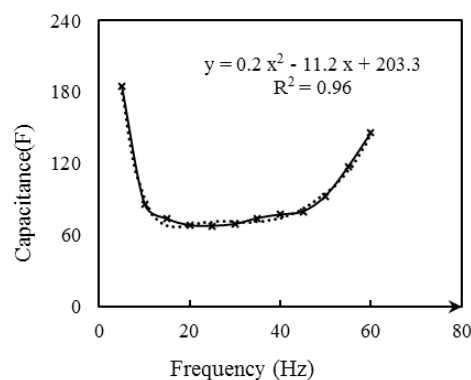


Figure 5d

Figure 5. (a) Capacitance modelling of *Pinus densata* at frequency between 10-50 Hz; (b) Capacitance modelling of *Pinus densata* at frequency between 50-120 Hz; (c) Capacitance modelling of *Pinus tabuliformis* at frequency between 5-60 Hz; (d) Capacitance modelling of *Pinus tabuliformis* at frequency between 60-120 Hz

hybrids have particular inclinations to maternal or paternal side in the kinship, instead of being evenly affected by each parent. Hybrid No.408 and 404 again have a closer position towards *P. densata*, in accordance with their capacitance values. The use of capacitance as an indicator for kinship among species may be related to inherent electrical properties of the basic genetic material sources.

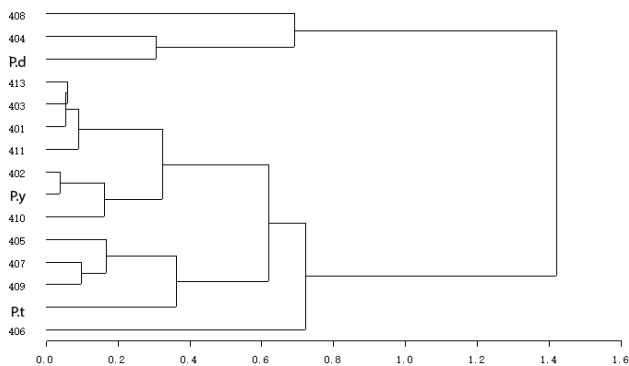


Figure 6. Capacitance clustering analysis of *Pinus densata* (P.d), *P. tabuliformis* (P.t) and *P. yunnanensis* (P.y) and the artificial hybrid (Hybrid)

### 3.4 Capacitance and Growth Adaptability Index

The water content and charged ions and particles are higher in seedlings in good growth conditions than that in poor conditions. Under external electric field, different capacitance of different plants might be due to their growth conditions. Therefore, we analyzed the correlation between capacitance and growth index in order to isolate the impact from growing environments. The growth adaptability index and capacitance data were measured at the same time.

To compare the capacitance and growth adaptability indexes (seedling height, survival rate, capping rate, germination rate, and mortality rate) of the species, we added capacitance value together regardless of the frequencies and calculated the average values for all indexes. The growth indexes were normalized to compare capacitance or electrical storage capacity and growth condition (Figure 7a). The capacitance of *P. densata* was consistent with its second-year growth height. The capacitance of *P. tabuliformis*, *P. yunnanensis* and the hybrid were consistent with their second-year seedling

survival rates, and the growth index of the hybrid and *P. tabuliformis* were the same. In addition to germination rate, mortality rate and capacitance were positively correlated for *P. densata*, *P. tabuliformis* and the hybrid (Figure 7b).

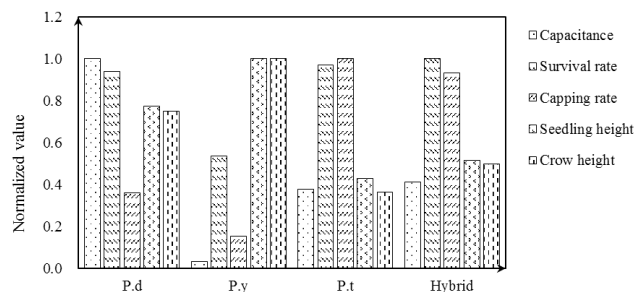


Figure 7a

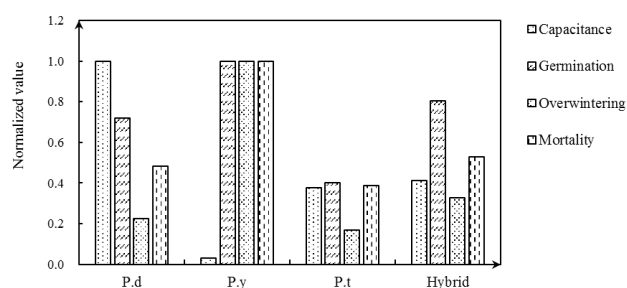


Figure 7b

Figure 7. (a) Normalized capacitance value and growth indexes of *Pinus densata*, *P. tabuliformis* and *P. yunnanensis* and the artificial hybrid; (b) Normalized capacitance value and mortality rate of *Pinus densata*, *P. tabuliformis* and *P. yunnanensis* and the artificial hybrid

The correlation coefficients between capacitance and second-year seedling height, second-year seedling crown height, and germination rate are negative (Table 1). Capping of height is an adaptation strategy for a seedling to store energy under low temperature and low photoperiod during winter. Capacitance and the capping rate was negatively correlated (Table 1), which is in agreement with previous findings<sup>[13]</sup>. In this study, capacitance is negatively correlated with mortality rate during winter and positively correlated to second-year survival rate.

#### 4. Discussion

The fluids between cells can be regarded as electrolyte<sup>[14]</sup> and when different current of low voltage was applied to plant tissues, the current was conveyed by the fluids. When the frequency of the current applied increases, part of the current will get into the cell membrane and conducted through the cell membranes of plant tissues<sup>[15]</sup>. The change of capacitance value of *P. densata*, *P. tabuliformis*, *P. yunnanensis* and the hybrid as the frequency changes indirectly reflects the properties of membranes. *Pinus densata* and the hybrids are likely to inherit more dielectric properties of membranes from the material parent *P. tabuliformis*.

Photosynthesis is the process to transfer light into chemical energy (formation of organic matter) by plants. When turning light energy into electrical energy, electrons are transferred between the two photosystems systems, PS II and PS I. Physiological function of photorespiration is to consume excess energy<sup>[15]</sup>. When the tested sample was applied to an electric field, the storage and consumption of capacitance are two indicators of electric field energy storage and loss, respectively. The capacitance of *P. densata* was the largest, indicating that *P. densata* had the largest capacity to store charges or electrical energy. It is known that visible light is an electromagnetic wave, and sunlight can be considered as field energy with a frequency range of 480-680 MHz<sup>[10]</sup>. When the frequency was greater than 15 Hz, the decline in the capacitance was the lowest in *P. densata*, indicating that the energy loss in *P. densata* was the lowest. Ma et al<sup>[13]</sup> also reported that the energy storage capacity of *P. densata* is much larger than *P. yunnanensis*, *P. tabuliformis* and the hybrids, and its energy consumption is the smallest among them.

The relationship between seedling growth indexes and capacitance describes the linkage between the biological properties and their dielectric properties. The capacitance and growth and adaptability index were similar between *P. densata*, *P. tabuliformis* and the hybrid, again indicating that maternal effect was greater than the paternal influence. This is consistent with previous findings, which showed that the characteristics of *P. densata* are similar to *P. tabuliformis* and *P. yunnanensis* but closer to that of *P. tabuliformis*<sup>[16]</sup>. High capacitance of *P. densata*

Table 1. Correlation analysis between growth parameters and capacitance

	Germination rate	mortality rate	2nd year survival rate	2nd year capping rate	2nd year seedling height	2nd year crown height	2nd year mortality rate
Capacitance	-0.189	-0.595**	0.537**	-0.072	-0.075	-0.076	-0.243

Notes:

\*  $P < 0.05$ , \*\*  $P < 0.01$

indicates high energy storage within the seedling and better adaptability to harsh environments, resulting in high survival rate and low mortality rate. *Pinus densata* has been reported to have a higher photosynthetic rate and a higher reproductive adaptation at high altitudes than its parental species<sup>[17]</sup>, achieving long-term survival under the harsh natural environment in Tibet, and eventually being able to evolve<sup>[18]</sup>. Two genotype of the first generation hybrids showed very close capacitance values as *Pinus densata*, and we could speculate that during natural selection, due to strong adaptability of the two, eventually they are able to survive and adapt to the environment.

## 5. Conclusions

We conclude that capacitance, a measure of energy storage capacity, can be used as a complementary indicator for studying genetic variation, adaptation ability to harsh environment, and kinship among species. In this study, we provide evidence to support that some particular genotypes of the artificial hybrid have a potential to evolve into *P. densata*, and the maternal parent has a stronger influence on the energy storage capacity of the hybrids. In addition, the ability of *P. densata* to adapt to harsh environment at a high altitude can be elaborated from its superior capacitance values over its parental species. This study opens a new perspective of using electrical parameters as a tool to study plant characteristics and provide complementary evidence of evolution and species kinship.

## Acknowledgement

This studied was supported by National Natural Science Foundation of China. We would also like to thank all those who helped with the experiment and the writing.

## References

- [1] Dong, X. Recent Progress and Challenges in the Study of Bioimpedance. Chinese Journal of Biomedical Engineering, 2008, 27(5): 641-643.
- [2] Ackmann, J.J., Seitz, M.A. Methods of complex impedance measurements in biological tissues. CRC Critical Review in Biomedical Engineering, 1984, 11(4): 281-311.
- [3] Macdonald, J.R. Impedance spectroscopy: emphasizing solid materials and systems. New York: John Wiley & Sons, Inc, 1992.
- [4] Schwan, H. The practical success of impedance techniques from an historical perspective. In: Riu PJ, Rosell J, Bragós R, Casas Ó, eds. Electrical bio-impedance methods: applications to medicine and biotechnology. Annals of New York Academy of Sciences, 1999, 873: 1-12.
- [5] Hao, Z., Zhang, G., Li, Y. Physiological and Biochemical Indexes and Electrical Impedance Spectroscopy of *Betula platyphylla* Seedlings under Heat Stress. Journal of Acta Botanica Boreali-Occidentalia Sinica, 2010, 9: 1844-1851.
- [6] Meng, Y., Zhang, G, Li, X., Gao, Z. Effect of NaCl Stress on Electrical Impedance Spectroscopy Parameters and Membrane Permeability of Moso Bamboo (*Phyllostachys edulis*) Seedling Leaves. Journal of Wuhan Botanical Research, 2010, 28(3): 341-346.
- [7] Guo, W., Wu, L., Wei, Y. Influence of water loss on physiological and electrical properties of plants. Journal of Northwest A & F University (Nat. Sci. Ed.), 2007, 35(04): 185-188.
- [8] Zhu, Z., Bao, Y. Study of the moisture content of soybeans on dielectric property of soybean. Journal of Zhejiang University (Agric.& Life Sci.), 2005, 31(02): 220-224.
- [9] Xie, C., Rao, K. Electromagnetic Field and Wave. Beijing: Higher Education Press, 2006.
- [10] Zhao, K., Chen, X. Electromagnetism. Beijing: Higher Education Press, 2003.
- [11] Qiu, G. Circuit. Beijing: Higher Education Press, 2006.
- [12] Gao, J., Wang, B., Mao, J.F., Ingvarsson P., Zeng, Q.Y., Wang, X.R. Demography and speciation history of the homoploid hybrid pine *Pinus densata* on the Tibetan Plateau. Molecular Ecology, 2012, 21(19): 4811-4827.
- [13] Ma, F., Chen, X., Li, Y. Study on loss spectrum parameters in *Pinus densata* Masters and artificial hybrid of *P.tabuliormis Carrière* × *P. yunnanensis Faranch*. Journal of Forest and Environment, 2016, 36(01): 1-6.
- [14] Pan, R. Plant physiology. Beijing: Higher Education Press, 2004.
- [15] Grimnes S., Martinsen O. Bio-impedance and Bioelectricity Basics. London, UK: Academic Press, 2000.
- [16] Kuan, C. Fundamental features of the distribution of Coniferae in Sichuan. Journal of Systematics and Evolution, 1981, 19(4): 393-407.
- [17] Ma, F., Xu, T., Chen, L. Zhang, X., Zhao, C. Functional Stability of Photosystem II in the Diploid Hybrid Species (*Pinus densata*) under Low Temperature Stress. Journal of Acta Botanica Boreali-Occidentalia Sinica, 2011, 31(6): 1174-1179.
- [18] Mao, J., Li, Y., Liu, Y., Hao, L., Wang, X. Cone and seed characteristic of *Pinus densata* and their adaptive fitness implications. Journal of Plant Ecology, 2007, 31(02): 291-299.