Kitano Tenmangu Shrine Sumitomo Forestry Co., Ltd.

Initiative to Protect our Precious Trees: Success in Propagating Sapling Using Tissue Culture from Sacred "Kitano Sakura" Cherry Tree, Follows on from Last Year's "Beniwa Kombai" Plum Tree Project

Flowers that change from white to pink, and a possible new variety

Sumitomo Forestry Co., Ltd. (President and Representative Director: Akira Ichikawa; Head Office: Chiyoda-ku, Tokyo; hereinafter "Sumitomo Forestry") and the Kitano Tenmangu Shrine (Chief Priest: Shigetoku Tachibana; Location: Kamigyo-ku, Kyoto) have been conducting research and development in relation to the propagation of the "Kitano Sakura" sacred cherry tree, located in front of the Kitano Tenmangu Shrine office, for the purpose of protecting and propagating the tree, a rare variety with flowers that change color as they bloom. On this occasion, Sumitomo Forestry has succeeded in using tissue culture, a biotechnological technique, to propagate saplings that will ensure this valuable cherry tree is passed down to future generations.

With flowers that change from white to pink after blooming, the Kitano Sakura is estimated to be more than 70 years old. After comparing the tree's genetic information against the Sumitomo Forestry cherry tree DNA database,* no exact match was found, so it is highly likely that this tree is a new variety.

* In March 2011, Sumitomo Forestry, the Forestry and Forest Products Research Institute, the National Institute of Genetics and the Association for Propagation of the Knowledge of Genetics jointly developed a technique for accurately identifying cultivated varieties of cherry trees utilizing DNA marker identification technologies. A DNA database was created of approximately 200 varieties of cherry tree, for which DNA samples were available, from among the 250-plus cultivated varieties of cherry tree apparently in existence. This database enables the accurate identification of cultivated varieties of cherry trees and, at the same time, enables clarification of the relationships between varieties with long histories.

Ukon no Baba at Kitano, also sung about in the Noh song "Ukon," once famous for its cherry trees

The Kitano Tenmangu Shrine was built in 947 as the grand head shrine for about 12,000 Tenmangu and Tenjinja shrines nationwide which deify Sugawara no Michizane. It is a place remembered in connection with Sugawara no Michizane, who has long been widely embraced as a god of learning.

Opened in 807 by Emperor Saga, the Ukon no Baba horse-racing grounds, located at the shrine, were a favorite place of the enshrined deity Sugawara no Michizane, the Major Captain of the Right Division of Inner Palace Guards, and are said to have been a popular area for cherryblossom viewing. The Noh song "Ukon," set on the



grounds of Ukon no Baba, is a typical song of spring celebrating the imperial reign, in which a goddess is the main character.

Cherry trees once planted along historic Odoi earthen embankment

Part of the ancient Odoi earthen embankment built by feudal lord Toyotomi Hideyoshi still exists at the Kitano Tenmangu Shrine. Ancient documents and other information at the shrine indicate that cherry trees were once planted along the Odoi embankment, with descriptions of the cherry trees within the shrine precincts being in full bloom. Unfortunately, they sustained profound damage in the 1934 Muroto typhoon and now only a few remain, with the Kitano Sakura thought to be one of them.



Bringing the scenes of the Noh song Ukon into the present

Background to propagation by tissue culture, and earlier developments

In order to prepare for the unexpected, such as the "plum pox virus" (PPV, also known as the plum ringspot virus), which has become prevalent around the world over recent years, and pass down the precious Beniwa Kombai sacred plum tree and other trees to future generations, the Kitano Tenmangu Shrine requested technical cooperation, for propagation of the tree by tissue culture, from Sumitomo Forestry with its successful track record in various arbor technologies concerning tissue culture and seedling production. At the same time, they also asked Sumitomo Forestry to help with propagation by tissue culture of the Kitano Sakura, an extremely unusual cherry tree. The purpose was to protect and propagate the tree because its health had started to decline due to the rapidly changing environment, including the effects of acid rain and global warming.

Seedlings propagated by one tissue culture method called the shoot-tip culture method (a technique using the tissue at the tip of the bud, called the "shoot apex") would supposedly experience "rejuvenation" (blastogenesis) relative to the age of the subject trees. From the perspective of protecting and preserving our precious trees and handing them down to future generations, this tissue culture method was selected and the project to propagate the Kitano Sakura was launched in 2014.

Sumitomo Forestry's Tsukuba Research Institute, which was involved in the research and development, conducted two years of experiments, based on literature and on its previous achievements in culturing cherry trees, plum trees and other plants, until achieving the current success.

Future initiatives: Using tissue culture for DNA identification of different varieties, and protecting the sights of Kitano for future generation

In addition to protecting the Kitano Sakura, propagating these seedlings using tissue culture will contribute to maintaining the culture of Kyoto. Going forward, Sumitomo Forestry and the Kitano Tenmangu Shrine will continue these efforts to protect and pass on the shrine's landscape to ensure generations to come can enjoy this aspect of Kyoto.

References:

Details of the sacred Kitano Sakura cherry tree

- Origin: unknown. Estimated age: 120 years. Height: approx. 8 m Diameter: approx. 2 m
- The core of the trunk is rotted away and a cavity is exposed, from close to the base to about the height of an adult's chest.
- Some roots are forming inside, but because they are exposed the health of the tree is in danger of decline.

Past activities of the Kitano Tenmangu Shrine

- 2009 Started propagating a sacred plum tree using tissue culture
- 2014 Started propagating a sacred cherry tree using tissue culture
- 2015 Successfully propagated the sacred plum tree using tissue culture, and named the new tree "Beniwa Kombai"
- 2016 Successfully propagated the sacred cherry tree using tissue culture, and named the new tree "Kitano Sakura"

Continuing to use tissue culture to propagate precious plum trees within the precincts of the Kitano Tenmangu Shrine

Overview of propagation technology using tissue culture

[workflow]

- (1) Collect winter buds, and from them use a microscope to extract only the meristematic tissue of the shoots (shoot apex).
- (2) Transfer the shoot apexes to test tubes, pour in a culture solution developed for the Kitano Sakura cherry tree, and cultivate to produce a large quantity of shoots (multiple shoots).
- (3) Grow long shoots from the multiple shoots by cultivating the multiple shoots in a solid medium.



Kitano Sakura in front of the shrine office with damaged trunk and branches

- (4) Divide the large quantity of long shoots into individual shoots, and transplant them into artificial compost with an added culture solution to encourage the shoots to take root. The shoots will take root in about 4 weeks, reproducing a whole plant (seedling). The steps to this point are conducted under sterile conditions.
- (5) Cultivate the seedlings in a greenhouse to acclimatize them to outside conditions (acclimatization process).



Photo 1 Multiple shoots (3rd month of culture)



Photo 2 Multiple shoots (6th month of culture)



Photo 3 Seedlings taken root in artificial soil (8th month of culture)



Photo 4 Saplings growing in ordinary soil