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For Immediate Release

Ninna-ji Temple
Sumitomo Forestry Co., Ltd.

—Ninna-ji Temple, Research Project—

Succeeded in Propagating Weeping Cherry Tree Seedlings Using Tissue Culture

Possible new variety to be christened “Yodozakura,” after the 49th head priest of Ninna-ji

The *Omurozakura* cherry blossoms at Ninna-ji Temple (Head priest: Yodo Minami, Ukyo-ku, Kyoto; hereinafter “Ninna-ji”) are located through the temple’s inner gate, on the left hand side. The *Omurozakura* are unique as they do not grow taller than the height of an average adult, and consequently their blossoms can be viewed at eye level. They are famous for being late blooming cherry trees that add the final touches of color to the end of Kyoto’s beautiful spring season. The *Omurozakura* of Ninna-ji are designated as a Historic Monument of Ancient Kyoto, and a UNESCO (United Nations Educational, Scientific and Cultural Organization; headquarters in Paris) World Heritage Site.

Among the *Omurozakura*, the last variety to bloom is one commonly known as the “weeping” cherry tree. It is believed that this variety does not grow anywhere other than at Ninna-ji. Moreover, there is only one weeping cherry tree on the grounds of Ninna-ji, making it extremely rare. The *Omurozakura* Research Project embarked on the propagation of seedlings to preserve this variety and to carry out a botanical investigation.

Sumitomo Forestry Co., Ltd. (President and Representative Director: Akira Ichikawa; Head Office: Chiyoda-ku, Tokyo; hereinafter “Sumitomo Forestry”) announced that Tsukuba Research Institute (Director: Naoteru Umesaki; Location: Tsukuba City, Ibaraki Prefecture), following its success with the *Omurozakura* (Omuro Ariake), proceeded with R&D related to the propagation of the weeping cherry tree and has successfully bred seedlings using a tissue culture method, which is a biotechnology technique to enable this precious variety to be passed on to future generations.

The *Omurozakura* are believed to have been planted in the third year of Shoho (1646), when the temple’s reconstruction was completed, are thought to be more than 360 years old. In light of the pronounced decline in the trees’ vitality in recent years, Ninna-ji consulted with the Cultural Properties Division of Kyoto and various other government agencies, aiming to unravel the mysteries of the growth of these cherry trees and to manage and maintain their scenic beauty. In April 2007, Sumitomo Forestry, in conjunction with the Graduate School of Horticulture, Faculty of Horticulture, Chiba University (Professor Eijiro Fujii), established the *Omurozakura* Research Project and has since conducted research, including growth surveys, soil and root system surveys, breeding of seedlings, and DNA analysis. In January 2010, the project was successful in the propagation of the Omuro Ariake, the main variety of *Omurozakura* cherry trees. The seedlings have reached a size where they can now be planted. The first Omuro Ariake seedling was planted on the grounds of Ninna-ji in February 2012. The project was also successful in reproducing seedlings for the weeping cherry tree. This is possibly a new variety and Ninna-ji plans to name it the “Yodozakura.”

■ ***Omurozakura* and the weeping cherry tree—a possible new variety—**

Omurozakura is the name given to the grove of cherry trees that was designated a famous scenic site on the grounds of Ninna-ji. There are several different varieties of *Omurozakura*, but over 90% of them are the Omuro Ariake variety. The Omuro Ariake variety consists of both single-petal and double-petal varieties. Among *Omurozakura*, which are known for being late-blooming, the weeping cherry tree is the last to blossom. Detailed research into the ecology of this tree has not been carried out thus far, so there are many questions about this variety of cherry tree. A comparison with a DNA database containing the DNA of around 200 varieties of cherry trees that was developed by the Forestry and Forest Products Research Institute and other

organizations, showed a clear DNA distinction with the other Ninna-ji cherry trees that were designated a famous scenic site and also with the other varieties contained in the database. This has led the project to believe that the weeping cherry tree is a new variety. This tree has been known as the weeping cherry tree until now, but the variety is scheduled to be officially named Yodozakura, after the Yodo Minami, who was head priest of Ninna-ji at the time of the discovery. Minami is the 49th head priest of the temple since it was established.

The major differences in appearance between the *Omurozakura* and the weeping cherry tree are that the branches and trunk of the *Omurozakura* grow upward while the branches of the weeping cherry tree extend sideways. Another difference is that the *Omurozakura* blooms from early to mid April, while the weeping cherry tree blossoms from mid to late April. In addition to the results of the DNA database comparison, the project will carry out surveys, including morphological observations of features such as flowers and leaves, and clarify in detail the differences between these two varieties.

* Forestry and Forest Products Research Institute, along with the National Institute of Genetics, an inter-university research institute corporation, the Association for Propagation of the Knowledge of Genetics, and Sumitomo Forestry, is developing a database by using a method it established in March 2011 to discern cultivars using DNA markers.

■ Reason for propagating seedlings using tissue culture

To produce the *Omurozakura* seedlings until now a method was used whereby a branch with roots is selected from epicormic branches and multiplied. The weeping cherry tree bears few if any epicormic branches making it impossible to divide the roots to grow more trees. It is believed this is why the propagation of seedlings was not carried out.

Generally, grafting techniques are used to propagate cherry tree seedlings. However, due to the lack of vitality of the weeping cherry tree, the branches generally do not grow much. Consequently, there are few good branches to choose from that would be ideal to use in grafting. Furthermore, the grafted seedlings are grown outdoors so there is risk of withering should they become damaged by insect infestation or disease. However, the development of culture conditions would allow the reproduction of many seedlings from a single bud using a tissue culture method. In addition, using this method, propagation is conducted in a sterile test tube so there is no concern of damage from insects or disease. Moreover, it is possible to preserve the seedlings semi-permanently by periodically changing the culture solution. This is believed to be an optimal method for passing on these valuable heritage trees to future generations.

Tsukuba Research Institute employed the shoot-tip culture method, a type of tissue culture, to develop a propagation technique for seedlings to preserve the rare weeping cherry tree variety for the future. In the shoot-tip culture method, the meristem of the bud (shoot-tip) is extracted under a microscope and reproduced. The shoot-tip, even in natural conditions, is said to be in a nearly aseptic (sterile) state, so there is little concern of bacterial infection. Also, this method is ground breaking, as insect or disease damage is not passed on to the seedlings even if the parent plant has been damaged by insects or disease. In addition, there is very little chance of mutation occurring using this method. Accordingly, there is a high likelihood that the traits of the weeping cherry tree will be passed on successfully.

■ Overview of propagation using the tissue culture method

- (1) Winter buds are collected and the meristem of the bud (shoot-tip) is extracted under a microscope.
- (2) The shoot-tip is transferred to a test tube and a culture solution, developed specifically for the weeping cherry tree, is added, and a vertical roll culture technique is used to produce, many shoots.
- (3) Buds are grown from the multiple shoots using a horizontal gyrator culture.
- (4) The numerous shoots that sprouted from the buds are separated individually and planted in artificial culture soil with a culture solution that promotes root growth. The shoots begin to grow roots in about 2 weeks, resulting in a perfect seedling. These procedures are conducted under aseptic conditions.
- (5) After about two weeks of low-temperature treatment, the seedlings are moved to a greenhouse to acclimatize to outdoor conditions (acclimation treatment).

There are no reported cases thus far of using tissue culture to propagate the weeping cherry tree so it was necessary to investigate growth conditions from the start. However, the Institute successfully reproduced the seedlings in about two years from the start of development, since the propagation conditions for the *Omurozakura* served as a reference.

■ Measures going forward

Sumitomo Forestry Landscaping Co., Ltd. (President: Kanpei Tokunaga; Head Office: Nakano-ku, Tokyo), a comprehensive landscaping specialist wholly-owned by Sumitomo Forestry, received an order in 2003 for the maintenance and management of the gardens at Ninna-ji. Caring for the trees on the temple grounds led to the current project. Tsukuba Research Institute took this opportunity to carry out research to develop methods to pass on the valuable heritage trees, *Omurozakura*, which had become notably weak, to future generations.

The reproduced seedlings are scheduled to be planted on the grounds of Ninna-ji. This will bring more color to Kyoto's spring and also continue to unravel the botanical mysteries surrounding the weeping cheery tree. Owing to the success of these projects, Sumitomo Forestry aims to continue to work with related parties going forward to carry out the maintenance and management of the temple gardens so that future generations can enjoy the *Omurozakura*.