Current Research Journal of Biological Sciences 5(4): 141-148, 2013
DOI:10.19026/crjbs.5.5479
ISSN: 2041-076X, e-ISSN: 2041-0778

Submitted: November 25, 2011 Accepted: November 08, 2012 Published: July 20, 2013

**Research Article**

# Preserve of Threatened Conifers (Cupressaceae) in Vietnam

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**Abstract:** Conifers in Cupressaceae distribute in forests at above 1000 m in elevation and are threatened by fragmented habitats and over-exploitation. Our results indicate that conifer population sizes were small (ca. 100 individuals) with low propagules, especially *C. lanceolata* var. *konishii*. The ISSR data showed low genetic diversity at both population and species levels, an average of 0.1025 and 0.0701 for *C. lanceolata* var. *konishii* and *F. hodginsii,* respectively (at population level) and 0.1357 and 0.1145 for *C. lanceolata* var. *konishii* and *F. hodginsii*, respectively as well. Genetic differentiation among populations per each species was high (Gst = 0.2771 and 0.22554 for *F. hodginsii* and *C. lanceolata* var. *konishii*, respectively). Factors that contribute to a reduction of low genetic diversities and high genetic differentiation and the priorities for species in Cupressaceae are discussed as well.

**Keywords:** *Cunninghamia lanceolata* var. *konishii*, *Fokienia hodginsii*, genetic diversity, ISSR markers, species conservation

## INTRODUCTION

Habitat fragmentation and destruction are the major causes of species extinction. They reduce population size, increase random genetic drift and inbreeding (Barrett and Kohn, 1991; Bijma *et al*., 2000). Consequently, there will be a reduction in genetic diversity, which might result in reduced fitness and increased susceptibility to environmental stochaticity (Van Treuren *et al*., 1993).

The conifer species (Cupressaceae) is an ancient group of seed plants. Conifers appeared over 300 million years ago and formed the dominant vegetation for long time. Conifers are characterized by naked seeds. Hermaphrodite cones do not occur and thus conifers are dioecious or monoecious. Outbreeding is frequently obligate. They are always wind-pollinated. According to Luu and Thomas (2004) eight Cupressaceae species were found in Vietnam where the species are restricted to highly fragmented habitats. Many species are restricted as *Cunninghamia lanceolata* var. *konishii* in adjoining area of Laos, *Xanthocyparis vietnamensis* in Ha Giang (Quan Ba), *Taiwania crytomerioides* in Lao Cai (Van Ban), *Glyptostrobus pensilis* in Dac Lac (Krong Nang, Ea H'leo), *Cupressus tonkinensis* in Lang Son (Huu Lung), but there are a few widespread species as *Calocedrus macrolepis* and *Fokienia hodginsii* in central and northern Vietnam.

Because of commercial value, conifers are over-exploited. Moreover, in recent years, increased human pressure has resulted in a great reduction in forest areas and increased level of fragmentation of surviving forests. All the species in Cupressaceae are threatened at global and national levels. Althought some populations are protected objectives in nature reserves, they are still in danger of extinction. In order to preserve the species in Cupressaceae, at present, we have a serious lack of information on genetic variation at population and species levels, especially the deleterious effects of human activities. These are very difficult to promote the conservation and sustainable use of the species. The objective of this study was to use ISSR markers to address Protection Forestry Department to stress the value of conservation. The study focused on two threatened conifers, *Fokienia hodginsii* and *Cunninghamia lanceolata* var. *konishii* in northern and central Vietnam.

## MATERIALS AND METHODS

**Study species:** Two studying conifers *Fokienia hodginsii* and *Cunninghamia lanceolata* var. *konishii* (Cupressaceae) occur in Vietnam. Luu and Thomas (2004) identified areas for the conifer *Fokienia hodginsii* in north and central Vietnam. While remaining species *Cunninghamia lanceolata* var.

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*konishii* is recorded in central Vietnam (adjoining areas of Laos). They occur in pure stands on top ridges of Limestone Mountains or closed evergreen tropical seasonal mixed submontane and montane forests on granite at more 900 m elevation. Cones mature by late October-December.

**Study areas and field works:** The research was carried out in Lao Cai: Den Thang, Hoang Lien Son National Park (22°09'-22°30'N and 103°30'-104°50'E, 1500-3000 m elevation) and Liem Phu secondary forests (22°-22°2'N and 104°2'-104°4'E, 300-1700 m); Ha Giang: Cao Bo secondary forests (22º45'-22º50'N and 104°48'-104°55'E, 870-2000 m) and Thai An secondary forests (22°58'-23°07'N and 105°04'-105°09'E, 1400-2100 m); Hoa Binh: Pa Co, Hang Kia-Pa Co Nature Reserve (21°30'N and 104°52'E), 1000-1200 m); Son La: Co Ma Nature Reserve (21°17'-21°25'N and 103°32'-103°44'E, 1000 -1816.8 m); Nghe An: Khe Thoi, Pu Mat National Park (18°46'-19°19'N and 104°31'-105°03'E,) for *Fokienia hodginsii* and Tay Son secondary forests (19°17'-19°26'N and 104°02-104°12'E, 1800-2216 m), Tam Hop, Pu Mat National Park (19°06'N and 104°21'E, 1200 m); Thanh Hoa: Bat Mot, Xuan Lien Nature Reserve (19°52-20°02'N and 104°58'-105°15'E, 800-1605 m) for *Cunninghamia lanceolata* var. *konishii*.

Sites were chosen to represent a range of disturbed habitats for each studied species. In general, their habitats are diverse in relation to levels of human disturbance. They include little disturbed forests in Khe Thoi of Pu Mat National Park, Ta Van of Hoang Lien Son National Park and Bat Mot of Xuan Lien Nature Reserve, disturbed forests in Cao Bo, Thai An, Liem Phu, Pa Co, Ky Son and Tam Hop, plantations in Liem Phu, Co Ma and Tay Son.

**Geo-morphology and climate:** On the geo-morphology, the areas consist of mountains with various elevations. The mountains are the result of sedimentation and intrusion. The marble and carbonate rocks exhibit a Karst style of weathering. Due to the very high humidity and rainfall in the areas, chemical weathering is prevalent. This is reflected in the clay nature of the soils. The areas in Hoang Lien Son, Hang Kia-Pa Co and Pu Mat mainly consist of limestone hills with high steep. Some sites were almost inaccessible on foot. No soil or little soil is covered on limestone hills. The valley between limestone hills is rather flat and wide and has been terraced for planting of wet rice and vegetable. Soils in Liem Phu are derived from alluvial deposits and tufa. Soils in Co Ma, Xuan Lien, Tay Son and Tam Hop are red-yellow and yellow feralite.

The climate of an area is reflected to its geographical features with tropical humidity and the ASEAN monsoons reflecting it. Due to influence of relief, the climate in each study area is mainly characterized by high rainfall and humidity. It is cold and dry in winter; hot and wet in summer. Mean annual temperature is 15.4°C in Sa Pa (Ghazoul and Chan, 1994), 19.1°C in Hang Kia-Pa Co, 19°C in Co Ma, 22.7°C in Ha Giang, 23.3°C in Xuan Lien and 23.6°C in Tuong Duong (Van *et al*., 2000) and 23.6°C in Pu Mat. The coldest month appears in January with mean temperature of 15.4°C, 16.5°C and 17.0°C in Ha Giang, Xuan Lien, Tuong Duong and Con Cuong, respectively; and hottest appears in June or July with the temperature of over 28°C for all studied areas. The annual rainfall averages from about 1268.3 mm in Tuong Duong to 3000 mm in Van Ban; and concentrates in summer months with 70% in Co Ma, Pu Mat and 85% in Thai an, Xuan Lien.

**Population size:** Although some individuals in a population were very difficult to reach on foot, especially *Fokienia hodginsii* in Hoang Lien Son National Park (Lao Cai), Cao Bo (Ha Giang) and a number of individuals per population was recorded during the surveys. Each individual was determined diameter (dbh). Tall in some young individuals was determined as well. These are useful to determine population structure and relationship between them and genetic parameters.

**Habitats:** Habitat description in the study areas included information on a list of vegetation types and general structure of vegetation (stratification, percent canopy cover, frequency of large trees, understory and abundance of ground and shrub cover). The climate (details of weather, abundance of rainfall, temperature and humidity) in the areas was recorded.

**Interviews:** Local people in all the study areas were interviewed about their jobs, incomes in relation to conifer exploitation. Management and plantation were also recorded during surveys.

**Survey site determination:** The GPS receiver was used to obtain information on survey sites. 1:50,000 map series were also used to provide area measurements of the study site and locations.

**Laboratory works:**

**Sample collection for DNA analysis:** 504 sample individuals were used from the 12 known populations: 8 for the *Fokienia hodginsii* populations and 4 for the *Cunninghamia lanceolata* var. *konishii* populations. The collected samples were wrapped by marked aluminum paper and placed in liquid nitrogen. They were transferred to the Laboratory of Molecular Biology, Institute of Ecology and Biological Resources and subsequently, stored at - 76°C until ready for use in DNA extraction. The samples were identificated on basis of past taxonomic treatments of collected specimens from these populations.

**DNA extraction:** Genomic DNA was extracted from young leaves or inner barks using the modified CTAB method by Xavier and Karine (2000). About 100mg of leaves or inner barks were ground in liquid nitrogen. Subsequently, the extraction buffer consisting of 640 μL of CTAB extraction buffer (100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl and 0.2% β-mercaptoethanol) and 160 μL of 10% CTAB was added and the mixture was incubated at 60°C for 1 h (leaves) or 3 h (inner barks). Then, 500 μL phenol:chloroform:isoamylalcohol (25:24:1) was added to mixture gently for 5 min to form an emulsion and centrifuged at 10,000 g for 6 min. DNA was precipitated by adding 2/3 volume of cold isopropanol solution and refrigerate for 20 min to the supernatant. The DNA pellet was washed with 200 μL of 5 M ammonium acetate and 600 μL of absolute ethanol, dried by air pump and dissolved in TE buffer (10mM Tris-HCl pH 8.0 and 1mM EDTA pH 8.0) with 1 μL RNase (1 μg/mL) per 100 μL DNA. The concentration of total DNA was determined using a fluorometer.

**DNA amplification for ISSR:** Polymerase Chain Reaction (PCR) was carried out in 25 μl solutions consisting of 2.5 μL reaction buffer, 2.5 μL MgCl2, 2 μL dNTP and 0.1 μL of primer, 1.25 units Tag DNA polymerase (Invitrogen) and 1.5 μL of template DNA. A total of eight ISSR primers were used in this study: Ubc810 (GAG AGA GAG AGA GAG AT), Ubc811 (GAG AGA GAG AGA GAG AC), Ubc815 (CTC TCT CTC TCT CTC TG), Ubc813 (CTC TCT CTC TCT CTC TT), Ubc835 (AGA GAG AGA GAG AGA GYC), Ubc836 (AGA GAG AGA GAG AGA GYA), Ubc840 (GAG AGA GAG AGA GAG AYT), Ubc841 (GAG AGA GAG AGA GAG AYC) and Ubc857 (ACA CAC ACA CAC ACA CYG). The reaction mixture was subjected to amplification in the Gene Amp PCR System 2400, under the following thermal cycler: an initial denaturing step at 94°C for 4 min, followed by 35 cycles consisting of 1 min at 94°C, 30s annealing temperature for each primer and 1 min extension at 72°C and 10 min at 72°C for a final cycle to complete the extension of any remaining products before holding the samples at 4°C until analyzed. The amplification products were separated by electrophoresis on 7.5% polyacrylamide gels in 1 x TAE buffer and then stained by ethidium bromide for 10 min. The banding patterns were visualized under UV light and photographed using a MEGA 8.4 Panasonic camera. 1 kb ladder was used as DNA standard (Invitrogen).

## DATA ANALYSIS

**Genetic diversity analyses:** ISSR bands were scored as presence (1) or absence (0). The binary data were analysed by PopGene v.1.31 (Yeh and Boyle, 1999) to estimate genetic diversity parameters: the proportion of polymorphic loci (P), the Nei (1973) gene diversity (H), the Shannon's index (I) (Lewontin, 1972).

Genetic diversities within and among the populations were analyzed for each polymorphic locus using (Nei, 1987) genetic diversity statistics: the total genetic diversity (Ht), the genetic diversity within populations (Hs), the coefficient of genetic diversity (Gst). The genetic differentiation among populations was estimated from allele frequencies using (Nei, 1972) genetic distance and identify for all pairs of populations. UPGMA cluster analysis of genetic distances was generated to examine genetic associations among populations or among individuals within populations using (Nei, 1972) genetic distance. The gene flow between populations (Nm) was also determined using Gst value: Nm = 0.5 (1-Gst)/Gst (Mcdermott and McDonald, 1993).

## RESULTS AND DISCUSSION

Luu and Thomas (2004) identified areas for two conifer species, of these, species, *Fokienia hodginsii* in northern and central Vietnam and the remaining species, *Cunninghamia lanceolata* var. *konishii* in central Vietnam, near Viet-Laos border. Two conifers occur in canopy in montane evergreen forest on derived soils over limestone, schist or granites. They were also cultivated mixture in secondary forests in Liem Phu (Lao Cai) and Tay Son (Nghe an). *F. hodginsii* were cultivated in Tay Son gardens. The geographical distribution of conifers is greatly influenced by human activities. Their distribution is highly fragmented. All populations are known to be very small for their survival in nature.

Data available from the spatial studies for all studying populations of two conifer species, *Fokienia hodginsii* and *Cunninghamia lanceolata* var. *konishii* indicated that a total number of conifers were small in all the studying populations. It estimates for *Fokienia hodginsii*, about 50 individuals at Cao Bo and 150 Thai secondary forests, below 100 at Bat Mot and Den Thang, the little disturbed forests, inside National Park of Xuan Lien and Hoang Lien Son, respectively. Similarly, for *Cunninghamia lanceolata* var. *konishii* population size averages 100 individuals at Khe Thoi and Bat Mot, little disturbed forests, inside Pu Mat National Park and Xuan Lien Nature Reserve. Wild population sizes at secondary forests of Tam Hop and Tay Son are smaller (below 100) in relation to exploitation from local people for their building and other purposes. Conifers in each population grow scatterly on flanks or ridges of hills. We observed rarely *F. hodginsii* at Sin Chai and Cat Cat, inside Hoang Lien Son National Park.

The large populations for *F. hodginsii* were cultivated from seedlings that collected in forests in Tay Son and Liem Phu secondary forests in 1980s according to the 661 programme with about several thousand individuals per site. One population of *C. lanceolata* var. *konishii* was cultivated from seedlings that collected in forests at Tay Son in 2001 according to this programme with about 500 individuals.

Demographic data from all studying populations showed the number of propagules produced by females in a wild population varied. This depended on environmental conditions and their habitats. Low proportion of propagules (natural regeneration, ≤5 cm in diameter) was determined 5% at Cao Bo secondary forest for *F. hodginsii*. Higher values were found for *F. hodginsii* populations in Thai an (27.5%) and Den Thang (55.1%). We have not observed natural regeneration in the little disturbed forests in Khe Thoi and Bat Mot and Tay Son secondary forests for *C. lanceolata* var. *konishii* and *F. hodginsii*. However, we observed natural regeneration on landslips in Tam Hop along the Viet-Laos road. These may be relative to age structure within populations.

At the population level, the proportion of Polymorphic loci (P) ranged from 29.41% (Bat Mot) to 46.47% (Liem Phu), an average of 33.82% for *F. hodginsii*, whereas these values ranged from 40% (Khe Thoi) to 58.26% (Tay Son), an average of 49.35% for *C. lanceolata* var. *konishii*. The gene diversity (H) ranged from 0.0635 (Ta Van) to 0.0828 (Pa Co), an average of 0.0701; and the Shannon's index (I) ranged from 0.1053 (Ta Van and Cao Bo) to 0.1309 (Pa Co), an average of 0.1145 for *F. hodginsii*. Similarly, these values were H = 0.1025, ranging from 0.0898 (Khe Thoi) to 0.1178 (Tam Hop) and I = 0.1651, ranging from 0.1436 (Khe Thoi) to 0.1867 (Tam Hop) for *C. lanceolata* var. *konishii* At species level, P, H and I were 98.82%, 0.0926 and 0.1765, respectively for *F. hodginsii*, while these values were 97.39%, 0.1357 and 0.2355, respectively for *C. lanceolata* var. *konishii*.

The mean genetic diversities of differences among individuals within populations (Hs) were 0.0701 and 0.1025 for *F. hodginsii* and *C. lanceolata* var. *konishii*, respectively. Similarly, the total genetic diversities (Ht) were 0.0970 and 0.1377 for *F. hodginsii* and *C. lanceolata* var. *konishii*, respectively. The Genetic differentiations (Gst) were found at 0.2771 and 0.2554 for *F. hodginsii* and *C. lanceolata* var. *konishii*, respectively. And gene flows (Nm) among populations were also found at 1.3046 for *F. hodginsii* and 1.4575 for *C. lanceolata* var. *konishii.*

Genetic identities and distances obtained from all pairwise comparisons of populations were presented. All identities obtained in comparisons of eight *F. hodginsii* populations and that of four *C. lanceolata* var. *konishii* populations exceeded 0.9. However, these values were lower for *C. lanceolata* var. *konishii* compared with *F. hodginsii*. Identities averaged 0.9677, ranging from 0.9538 (Cao Bo and Co Ma) to 0.9855 (Liem Phu and Ta Van) for *F. hodginsii*. This value was 0.9478, ranging from 0.9285 (Tam Hop and Khe Thoi) to 0.9657 (Tam Hop and Tay Son) for *C. lanceolata* var. *konishii.* The mean genetic distance between populations for each species were low, 0.0336 (0.0146-0.0473) and 0.0537 (0.0349-0.0742) for *F. hodginsii* and *C. lanceolata* var. *konishii*, respectively.

In this study, low genetic diversity within populations and high genetic differentiation among populations were found for two studying species, *F. hodginsii* and *C. lanceolata* var. *konishii* in Vietnam. These indicated that major factors threatening the survival of these conifers are related to human activity. These conifers were damaged by human beings through overexploitation for commercial reasons, destruction of habitats. The small population size (<100 individuals/population), fragmented habitats and low reproduction ability may result in low levels of genetic diversity within and gene flow among populations; and high genetic differentiation among populations.

The ISSR markers were used to assess genetic diversity measures within and among populations of *F. hodginsii* and *C. lanceolata* var. *konishii* in this study. Both population and species levels have lower values of genetic diversities than do other coniferous species. High values of genetic variability were reported for populations of many conifers such as *Pinus strobus* P = 47.8% and H = 0.195 (Rajora *et al*., 1998), *Pinus pinceana* P = 56.5%, H = 0.174 (Ledig *et al*., 2001), *Pinus brutia* P = 68%, H = 0.271 (Korol *et al*., 2002). Low genetic variabilities have been also found in some conifers possessing restricted occurrences: *Abies sibirica* P = 20% H = 0.0642 (Larionova *et al*., 2007), *A. flinckii* P = 30.2%, H = 0.113, *A. guatemalensis* P = 20%, H = 0.069, *A. hickeli* P = 28.2%, H = 0.1 *A. religiosa* P = 31.8%, H = 0.108 (Aquirre-Planter *et al*., 2000), *A. lasiocarpa* P = 43.4% H = 0.124 (Shea, 1990), *Picea breweriana* P = 44.2% H = 0.129 (Ledig *et al*., 2005). In another studies, high levels of genetic variability within and among conifers were also obtained: *Picea sitchensis* Hs = 0.147 and Ht = 0.159 (Yeh and El-Kassaby, 1979), *Pinus longaeva* Hs = 0.465, Ht = 0.484 (Hichert and Hamrick, 1983), *P. rigida* Hs = 0.147, Ht = 0.152 (Guries and Ledig, 1982). Our results confirm the suggestion that the genetic structure of natural populations of *F. hodginsii* and *C. lanceolata* var. *konishii* is strongly affected by small population sizes. The number of observed individuals was small and varied considerably, with *C. lanceolata* var. *konishii*, averages 100 individuals at Khe Thoi and Bat Mot, little disturbed forests, inside Pu Mat National Park and Xuan Lien Nature Reserve. Wild population sizes at secondary forests of Tam Hop and Tay Son are smaller (below 100) in relation to exploitation from local people for their building and other purposes. While population sizes are about 50 individuals at Cao Bo and 150 Thai An secondary forests, below 100 at Bat Mot and Ta Van, the little disturbed forests, inside National Park of Xuan Lien and Hoang Lien Son, respectively for *F. hodginsii*. Such small populations are the occurrence of inbreeding and the effect of genetic drift (Harl and Clark, 1989; Barrett and Kohn, 1991; Ellstrand and Elam, 1993). The current distribution of *F. hodginsii* and *C. lanceolata* var. *konishii* has been strongly influenced by fragmented habitats. The species occupies in the forests at 1300-1900 m elevation. Forests have been greatly fragmented by human activities and formed small forest patches. A few natural populations of these species remain in such small patches. Forests were destroyed and converted other landscape unsuitable for *F. hodginsii* and *C. lanceolata* var. *konishii* survival. In addition, the low level of genetic variability in this species might be caused by founder effects associated with altered climatic conditions. The logging activity and the associated creation of gaps cause of change in original vegetation structure. There was variation in the spatial distribution, age class structure of trees and the invasion of exotic species. *F. hodginsii* and *C. lanceolata* var. *konishii* distribution is characteristic terrain and climate. Therefore, the species has been exposed to geographic isolation. The results also showed that genetic parameters made on the adult trees were lower than those made on the seedlings at both Tay Son and Tam Hop for *C. lanceolata* var. *konishii*. It is related to disperse on large distances. The seeds are dispersed by wind. *C. lanceolata* var. *konishii* is canopy trees that reach at least 0.5 m dbh and 41 m height at Tay Son and Tam Hop.

A limited genetic variability within populations also indicated considerable levels of differentiation among populations. Estimates of the Gst value for *F. hodginsii* and *C. lanceolata* var. *konishii* populations showed high amounts of genetic differentiation (Gst = 0.2771 and 0.2554, respectively). This value was clearly higher than those reported in other coniferous species, such as *Pseudotsuga menziesii* Gst = 0.026 (Yeh and O'Malley, 1980), *Pinus longaeva* Gst = 0.038 (Hiebert and Hamrick, 1983), *Pinus sibirica* Gst = 0.041 (Goncharenko *et al*., 1993), *Pinus monophylla* Gst = 0.033 (Hamrick *et al*., 1994), *Pinus albicaulis* Gst = 0.034 (Jorgensen and Hamrick, 1997), *Pinus flexilis* Gst = 0.101 (Jorgensen *et al*., 2002). In another cases, the Gst value detected was higher for *Pinus attennuata* Gst = 0.24 and *P. muricata* Gst = 0.29 (Wu *et al*., 1999); *Pinus brutia* Gst = 0.29 in the Marmara region and Gst = 0.35 in western Mediterranean region (Kandermir *et al*., 2004), *Picea asperata* Gst = 0.34 (Xue *et al*., 2005). The result confirms the assumption that genetic drift increased genetic differentiation among populations (Ellstrand and Elam, 1993). The high differentiation could be a consequence of limited gene flow (Nm = 1.4575 for *C. lanceolata* var. *konishi*i and 1.3046 for *F. hodginsii*). Fragmented habitat was gene flow barriers and decreased migration among populations for *C. lanceolata* var. *konishii* and *F. hodginsii*. Founder effects might contribute to the high level of genetic differentiation among the populations.

The destruction of suitable forests has been the major cause of the number decline in conifer populations associated with a rapid increase of local human population and economic development in recent years. Human activities included cleared forests for cultivation and settlements, exploitation for commercial purpose, firewood and building. Consequently, forests in areas where conifers were recorded have been greatly reduced and fragmented. They are to remain the principal threat to the species, because of converted habitat unsuitable for their survival. Moreover, due to high commercial value, conifer species were overexploited and has disappeared in many areas. All known populations of conifer outside protected areas, even those inside are at risk of being eliminated. At present, some conifer species is only found in wild on flanks and ridges of the limestone hills in restricted areas in Bat Dai Son Nature Reserve (Ha Giang) for *Xanthocyparis vietnamensis*, Liem Phu (Lao Cai) for *Taiwania cryptomerioides*, while *Fokienia hodginsii* is found on derived soils over limestone or granites in Lao Cai, Ha Giang, Thanh Hoa and Nghe An. Similarly, *Cunninghamia lanceolata* var. *konishii* is also found in the little disturbed forests, near border Viet-Laos in Nghe An and Thanh Hoa. Their survival is dependent mainly on human activities. Some major threats to the populations are discussed here.

**Habitat loss:** For many years, human activity in tropical forests occurred by the conifers has influenced the natural vegetation such that its cover has been greatly reduced and fragmented. A number of smaller forest patches are isolated with each other. Fragmentation of original forest is related to clearance for both cultivation and settlements. For these reasons, they remain the principal threat to most species, because of converted habitat unsuitable for their survival. Most forests close to all villages and along the roads to the villages through study sites inside and outside protected areas are converted to the cultivation. This process is not a recent phenomenon; but it appeared in the past with more intensive than at present. Forest clearance rates may be high in all villages, especially Y Linh Ho, Sin Chai, Seo Mi Ti (inside Hoang Lien Son National Park), Liem Phu (outside Hoang Lien Son National Park), Cao Bo (Ha Giang) and Tay Son (Nghe An). Due to traditionally practicing of shifting cultivation from farmers, after two or three years cleared land is cultivated unsuitainable and abandoned. Moreover, an increase of population pressure led to limited area of accessible land.

Consequently, cleared land tends to be on steep slopes. Gingers were planted along side stream up to above 2000 m elevation (Cat Cat, Seo Mi Ti and Cao Bo) under the forest canopy after removal of all understorey trees and the ground layer together with some big trees of the canopy. After some ten years, some areas of cleared and abandoned land for villages appeared secondary forest and plantation. However, fuelwood collection and animal husbandry also degrade forest types.

**Logging:** The logging activity removes most large, old trees from forests and thus, causes a change in the vegetation structure. This alters the spatial distribution and age structure of trees in vegetation. Therefore it will affect the patterns of effective pollen dispersal and hence genetic structure of subsequent generations (Kathrin and Malcolm, 1991). Logging can be considered as the main activity for all farming households in the studying area. However, in recent years, logging intensity has decreased in protected areas, especially in the National Park of Pu Mat and Hoang Lien Son and Xuan Lien Nature Reserve in relation to understanding of local people on biodiversity conservation, their living standard and management of local authority. Cutting wood trees to earn money is a pressure felt in all villages within and outside a forest. Logging is often carried out during dry season (November-January). Species in Fagaceae and other families were also removed. The wood from *Fokienia hoginsii* is very available for export, furniture and building. It was sold for about 3 million dong (Vietnam current) in the Sa Pa market. After cutting down trees, local people cut the tree into blocks with size 0.2 m x 0,2 m x 2.5 m each other in the forests and transfer them outside the forest by foot. In the fast, logging activity took place on large area, around the villages (Seo Mi Ti, Sinh Chai and Cat Cat), Van Ban, Cao Bo and Tay Son where *F. hodginsii* was abundant. Enterprises were also implemented exploitation of *F. hodginsii* in Liem Phu, Cao Bo, Tay son in 1990s. Logging appears to be increasing in the area at higher altitudes when in lower areas, *F. hodginsii* is scarce. There are many evidences for exploitation in Thai An, Liem Phu, Sa Pa, Cao Bo and Tay Son. There were about 10 sites to be logged on each studying area. One group of 5 people from Thai An or Cao Bo with piting-saw seen during the survey. Some noise from logging activity was heart at 1700 m in Thai An, Cao Bo and Seo Mi Ti. For *C. lanceolata* var. *konishii*, the wood is very available for casket and building. We observed all houses in Tay Son village made from this wood. Although a logging is banned in protected areas, there is still evidence on this activity, especially in Xuan Lien Nature Reserve and Hoang Lien Son National Park. Many woods from local people are kept at Guard station.

**Fire:** Fire is one of threats to conifer populations in relation to man-made during clearance of forest land for cultivation or through forest campfire neglect. We found this evidence. According one local people, one population of *F. hodginsii* was fired in 1999-2000 in Seo Mi Ti (Hoang Lien Son National Park).

In conclusion, two conifers, *F. hodginsii* and *C. lanceolata* var. *konishii* are seriously threatened and urgent action could be required to preserve the species and their habitats.

They maintained low level of genetic variability and high level of genetic population differentiation. They are the results of human interference. *C. lanceolata* var. *konishii* and *F. hodginsii* habitats have been degraded and fragmented and only a few natural populations survived.

As mentioned results, the obvious management is to reduce inbreeding through maintenance of population numbers, encouragement of outcrossing and migration among populations for each conifer species. In order to conserve genetic resources of threatened conifers in Vietnam, several steps must be taken. The following recommendations are necessary to ensure in a long-term sustainability.

Forestry low enforcement should be strengthened so that logging and fire wood collection in forests where conifers living, cycad exploitation must be limited and prohibited. Department of Forestry Protection should establish contracts with local communities for the protection of conifers and their habitats. The contracts are signed for all areas of natural forests where conifers occupied. Bare and degraded land should be allocated for plantation and natural regeneration.

Ex-situ conservation of conifers could be immediately implemented. It will be a protective and managed site to grown conifers and prevent potential genetic erosion of wild conifer populations. Therefore, ex-situ collection will be the source material to re-establish as necessary to maintain conifers in long-term sustainability.

Training course should be provided for local people, even forest protection staff so that they understand conservation value of conifers and the sustainable management of forest resources.

The sale of conifers should be controlled at Tam Hop, Tay Son (Nghe An), Thai An (Quan Ba, Ha Giang) and other locations.

## ACKNOWLEDGMENT

This study is supported by Vietnam-Sweden Research Cooperation Fund (No 67-RF2).

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