Native habitats and salt stress responses of wild Hydrangea macrophylla

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Abstract

Hydrangea shrubs, developed from wild *Hydrangea macrophylla* endemic to Japan, are very important plants for landscaping and horticulture. *H. macrophylla*, likely speciated from *H. serrata* growing in the mountainous zone, have migrated into coastal zone. However, there is still a lack of information about the habitats of wild *H. macrophylla* and its adaptative responses to salt-stressed coastal environments. Therefore, in this study we conducted a survey of wild *H. macrophylla* populations and its native habitats. The results of the habitat survey showed that *H. macrophylla* is mainly distributed in coastal zones that have a saline atmosphere, triggered by high wind speed, high soil $EC_{1:5}$ and large soil sodium contents, capable of causing severe damage to many plant species. Additionally, we confirmed some inland populations in a part of the Izu Peninsula and on the Izu islands. Several of these inland *H. macrophylla* populations inhabited mountain vegetation areas favored by *H. serrata*, and had thinner leaves than the coastal populations. We also found *H. macrophylla* individuals growing in oligotrophic environments such as on rocky surfaces and in volcanic ash scoria. Leaf cation analysis suggests that *H. macrophylla* adapts to salt stress by regulating Na^+ allocation and preventing high leaf Na^+ concentrations. The findings of this fundamental investigation of native habitats and adaptation to coastal zone should help facilitate further studies on *H. macrophylla* ecosystem, abiotic stress and conservation.

Introduction

Hydrangea macrophylla (Thunb.) Ser. f. *normalis* (E. H. Wilson) is an endemic species of Japan and distributed in the following limited locations; the Boso, Miura and Izu Peninsulas of the Kanto region in Honshu, and the Izu and Bonin islands located south of the Izu peninsula (Iijima et al. 1958, Takayama 2019; Kato et al. 2020) (Fig. 1). *H. macrophylla* is a deciduous plant with large shiny leaves and beautiful flowers and mainly inhabits coastal zone which are exposed to various abiotic stresses, especially salt stress, and thus will have strong resistance against abiotic stress. These characteristics make it popular plant for horticulture and landscaping not only in gardens but also coastal areas as well as abiotic-stressed roadside in urban areas. Therefore, there is likely to be increased demand for *H. macrophylla* as an important genetic resource. However, while various studies have been conducted on ornamental hydrangeas originated from wild plants of *H. macrophylla* (e.g. Conolly et al. 2010; Niu et al. 2020), there have been limited studies on the native ecology and habitat adaptation of the naturally distributed wild plants.

H. macrophylla is thought to have speciated from *H. serrata* var. *serrata* (Uemachi et al. 2014), which grows naturally in the domestic mountainous areas. Therefore, *H. macrophylla* likely acquired abiotic tolerances as it evolved and migrated into coastal habitats. Previously, we investigated the morphological traits of *H. macrophylla* grown sympatrically in net chamber and found that *H. macrophylla* had thicker leaves and more wax development than *H. serrata*, indicating adaptation to abiotic environment of coastal zones (Morimoto et al. in press). However, there is little information about the ecology of the plants growing in their native habitats, nor how they have adapted to the different habitat conditions (soil EC and Na^+ contents, etc.), and saline atmosphere of coastal zone.

Therefore, this study aimed to assess the native habitats of *H. macrophylla* and investigate how the plants have adapted to different habitat conditions, especially the salt-stressed environments of the coastal zone. We used empirical ecological data of various *H. macrophylla* habitats that had been surveyed since 2013 for the conservation of genetic resources. Thus, we organized the ecological information based on the distribution, vegetation, and habitat characteristics gathered from the field survey. Then, we analyzed the Na^+ concentration in soils of the coastal habitats and the leaves from coastal populations to understand how the plants have adapted to Na^+ toxicity in coastal zone.

Materials and Methods

Field survey of native habitats

During the period from 2013 to 2022, we surveyed the habitats of wild plants of *H. macrophylla* in the following locations; the Boso, Miura, and Izu peninsulas and the Izu islands of Izu-Oshima, Nii-jima, Kozu-shima, Miyake-jima, Mikura-jima, Hachijo-jima, and Aoga-shima (Fig. 1). In the current study, we categorized the distribution and habitat characteristics of *H. macrophylla* based on these habitat surveys together with information from previous reports (Iijima et al. 1958; Oyama 1999, Takayama 2019; Kato et al. 2020) and a vegetation survey by the Ministry of the Environment (Biodiversity center of Japan, 2012). The maps for the habitat distribution were obtained from the Geospatial Information Authority of Japan (GSI) tool (https://maps.gsi.go.jp/). The altitudes and approximate distance of the native habitats from the sea were calculated using GSI analysis.

The mean annual rainfall and wind speed data for 30 years (1991 to 2020) were collected from the Japan Meteorological Agency (2021). The meteorological data from 2003 to 2020 were used for Nii-jima and Kozu-jima, while data from 2006 to 2020 were used for Kisarazu in the Boso Peninsula. For Aoga-shima, data from March 2021 to January 2023 were collected from the Mukaizawa weather station in the village of Aoga-shima (Aoga-shima village, 2011).

To investigate the adaptative responses to salinity in wild *H. macrophylla*, the populations on the Izu Peninsula, Izu-Oshima and Hachijo-jima were selected as sampling sites since these areas have a unique geography that includes high mountain, mountain vegetation, and desert zone, in addition to coastal zone (Fig. 2). To investigate leaf plasticity, cuttings were sampled on June 4, 2022 from two contrasting habitat environments on Hachijo-jima; Mitsune which is a coastal environment with lots of natural sunlight and Kashitate which is an inland habitat with mountain vegetation. The cuttings collected from both habitats were grown orthotopically for five months in a net chamber and greenhouse at Meiji University. The thickness of the leaves on the second node from the apical leaves was then measured by an MDC-25PX micrometer (Mitutoyo Co., Kanagawa, Japan).

Soil analysis

Details of the soil sampling date and sites are shown in Fig. 1, Fig. 2 and Supplementary Table S1. Soil samples were collected from locations beside where *H. macrophylla* was growing. In addition, soils were also collected from the native habitat area of *H. serrata* in Irisukawa, (Gunma prefecture), to compare with the habitat environment of *H. macrophylla* (Fig. 1). After removing the soil surface (approximately 1 cm thickness), the soil was taken from approximately 0–15 cm depth with falcon tubes. The pH, $EC_{1:5}$ and sodium ions of the soil samples were analyzed according to Samsone and Ievinsh (2018). First, the soil samples were completely dried at 80°C. Then, 200 mg of the dried soil was mixed with 500 µL of deionized water, and subsequently vortexed at 2,250 rpm for 30 min. The solution was incubated for 15 min and centrifuged at 5,000 g for 1 min. The pH (v/v = 1/2.5) of the supernatant was measured using a pH meter (LAQUAtwin-pH-33B, Horiba Ltd., Kyoto, Japan) pre-calibrated using standard buffer (pH 4.01 and pH 6.86). The supernatant was returned to the sample tube containing soil solution and added with 500 µL of deionized water. After vortex at 2,250 rpm for 15 min, the soil sample was centrifuged at 5,000 g for 1 min. The $EC_{1:5}$ and water-soluble Na^+ of the supernatant were then measured using an EC meter (LAQUAtwin-EC-33B, Horiba Ltd.) and a Na^+ ion meter (LAQUAtwin-Na-11, Horiba Ltd.), respectively. These meters were calibrated with the supplied kit standard solutions. The exchangeable Na^+ was measured by following procedure. The 50 mg of the dried soil samples was added with 1 mL of 1M ammonium acetate (pH 7.0), and subsequently vortexed at 2,250 rpm for 30 min. The soil sample was centrifuged, and the exchangeable Na^+ of the supernatant was measured using a LAQUAtwin-Na-11 (Horiba Ltd.) after pre-calibration was performed using sodium ion solutions (150 ppm and 2,000 ppm Na^+) created with 1 M acetic ammonium (pH 7.0) and NaCl.

Analysis of Na^+ and K^+ in leaves

Leaves were collected from sampling sites on Izu-Oshima and Hachijo-jima to measure Na^+ and K^+ leaf concentrations. The leaves were collected from Izu-Oshima on January 2022 (winter season) and from Hachijo-jima on June 2022 (summer season) (Fig. 2). The Na^+ and K^+ analyses were conducted on the leaves of the second or third node counting from the apical leaves. The petiole of the detached leaf was covered with parafilm, and the leaf was placed in a plastic bag. 10 mL of deionized water was applied to the leaf surface, and the mixture was shaken for 30 sec to wash off the attached salts.

Subsequently, the leaf blades without midribs were completely dried at 80°C for analysis of internal Na^+ and K^+ contents, according to Samsone and Ievinsh (2018), with some modifications. The dried leaves were crushed using pestle and mortar. Then, 25 mg of the powder was mixed with 1 mL of deionized water and two stainless-steel beads. The solution was vortexed for 1 hour and incubated for 15 min. Then, 700 µL of supernatant was moved to a microtube and centrifuged at 5000 g and 25°C for 5 min. The N^+ and K^+ of the supernatant were measured using compact ion meters (Horiba Ltd.), pre-calibrated using standard solutions. The concentration of Na^+ and K^+ was expressed as µmol/DWg.

Statistical analysis

Data were statistically analyzed using the EZR (ver. 1.41) software. Tukey's multiple comparison test was used to compare leaf thickness and cation contents in leaves between populations from different environments and locations. All data are shown as mean ± SE obtained from three biological replicates.

Results and Discussion

Distribution

H. macrophylla was distributed across large parts of the coastal zones of the Izu, Miura and Boso peninsulas in Honshu (Figs. 1A). The distribution was discontinuous, likely due to terrain and development of roads and buildings. On the eastside of Izu Peninsula, *H. macrophylla* was distributed from Atami in the north of the peninsula all the way to the southern end at Cape Irozaki. However, on the west side of the peninsula, the distribution was limited to the more southerly areas, only as far north as Iwachi (Fig. 1A). In addition, *H. macrophylla* was found in three inland areas of Izu Peninsula; Yukawa and Yahatano of Ito city, and Okawa of Higashi-Izu town, which were located ca. 900 m, 1.2 km and 1.6 km from the sea, respectively (Fig. 1A). The distribution was continuous from coastal to inland areas in these areas. the *H. macrophylla* plants in Yahatano and Okawa grew close to *H. serrata* plants, and several *H. macrophylla* individuals displayed thinner leaf thickness with less waxy than the other *H. macrophylla* growing in coastal zone (Fig. 3B). In Yukawa, there was a continuous distribution of *H. macrophylla* from 100 m – 900 m away from the coastline, and there were no *H. serrata* growing there. These surveys suggest that several *H. macrophylla* populations have migrated inland from the coastal zone.

H. macrophylla was also widely distributed on the nine inhabited Izu islands of Izu-Oshima, To-shima, Nii-jima, Shikine-jima, Kozu-shima, Miyake-jima, Mikura-jima, Hachijo-jima and Aoga-shima (Fig. 1B). Additionally, information from the Biodiversity Center of Japan (2012) and Oyama (1999), confirm *H. macrophylla* habitats on Jinai-jima, Tori-shima, and Hachijo-kojima, which have arid regions similar to Hachijo-jima. In the Bonin islands, *H. macrophylla* is distributed on Kita Iwo-jima and Minami Iwo-jima (Takayama 2019; Kato et al. 2020), but not on Iwo-jima (Fig. 1C). The distribution of *H. macrophylla* in the Izu islands has expanded to not only coastal zone but also to inland as well as partial areas of the Izu Peninsula (Fig. 3C, D). There are many populations inhabiting zones 100 to 500 m from the sea, which can be defined as semi-coastal populations. In addition, we also found many populations growing naturally among the mountain vegetation (Fig. 3D). These mountainous populations had apparently thinner and less waxy leaves than the coastal populations. However, when rooted cuttings of both the mountainous and coastal plants were grown in a net chamber and green house at Meiji University, some plants originating from the mountain population cuttings had leaves with similar thickness to the plants originating from the coastal zone cuttings (Fig. 4). This result suggests that there was some plasticity in leaf morphology between coastal and mountain vegetations. Therefore, it is possible that the coastal population migrated to inland zones after the speciation from *H. serrata* to *H. macrophylla* in the coastal zone.

Native habitats

Tolerance against abiotic stress can be affected by the meteorological environment. For example, in Taiwan it has been shown that rainfall which affects soil characteristics can cause variation in salinity tolerance of *Zoysia spp.* (Weng and Chen 2001). Also, *Setaria viridis* showed differences in salt spray tolerance in different environments, depending on various factors including differences in wind speeds in coastal and inland zones (Itoh 2021). In the current study, there were clear differences in the meteorological conditions in the various native habitats of *H. macrophylla*. The mean annual rainfall of the habitats of *H. macrophylla* ranged from 1,500 mm to 3,000 mm, with the Izu islands of Izu-Oshima, Miyake-jima, Hachijo-jima and Aoga-shima having higher rainfall than those of the other native habitats (Fig. 1). Additionally, it has also been found that the wind speed differed between the habitats, with especially higher wind speeds around Cape Irozaki and the Izu islands. This could expose coastal habitat plants like *H. macrophylla* to more salt spray than plants like *H. serrata* in mountainous habitats (Boyce 1954). Therefore, there could also be differences in the salt tolerance of *H. macrophylla* populations depending on the specific meteorological environment in each habitat.

In most of the *H. macrophylla* populations, the plants grew together with other coastal plants like *Crinum asiaticum*, *Pittosporum tobira*, and *Farfugium japonicum* (Fig. 3E). The proximity of the *H. macrophylla* to other plants suggests that the *H. macrophylla* might communicate with neighboring plants to help alleviate environmental stress, such as drought stress. For example, in the Ura-sabaku (desert) originated from volcanic ash scoria soil in Izu-Oshima, a few *H. macrophylla* were found growing together with *Rubus ribisoideus* (Fig. 2, 3F). Although these are arid soils, at about 50 cm depth the scoria soil had a moisture content of around 40%. Also, solitary *H. macrophylla* seedlings were found growing on rock cliffs of the coastal zone in Hachijo-jima (Fig. 3G), where the surface was constantly wet due to water flowing down from the top of the cliffs due to the nearby waterfall. Furthermore, we found several individuals growing like epiphytes attached to the stems of *Cyathea spinulosa* (Fig. 3H). This suggests that seeds carried by the wind were able to germinate due to the relatively high moisture content of the *C. spinulosa* stems. These findings suggest that *H. macrophylla* may be sensitive to drought and can adapt to oligotrophic environments. Recently, several studies have shown that similar close mixed-planting can enable plants improve tolerance against environmental stresses (Nanhapo et al. 2017; Yamane et al. 2018). Therefore, we consider that the close growth of *H. macrophylla* to other plants in most of the populations may help it to alleviate drought stress.

Adaptation to environmental stresses in native habitats

Most of the coastal habitats of wild *H. macrophylla* were located 10 to 40 m from the sea (Supplementary Table S1). In all these places, the plants were exposed to sea water, either directly from sea water splashing for the closest plants or from sea spray carried by strong coastal wind. The *H. macrophylla* growing in the coastal zone tended to show significant defoliation with shortened internodes (Fig. 6). Also, the mature leaves were hardened and wrapped around the young leaves, whereas the non-matured leaves were healthy and not hardened (data not shown). It is known that salt stress affects

morphological traits by shortening internode length (Okinaka et al. 1989; Valdez-Aguilar et al. 2009) and accelerating defoliation (Brumós et al. 2009; Kamiab et al. 2014). For example, in mangrove species salt is stored in older leaves that then fall (Kato et al. 1985). These findings suggest that *H. macrophylla* has similar morphological adaptations to salt stress, by defoliation and shielding young leaves from salt-spray damage by surrounding them with the mature leaves.

The soil pH of *H. macrophylla* habitats in the Izu Peninsula, Izu-Oshima and Hachijo-jima tended to be lower than those of the mountain habitats of *H. serrata* (Irisukawa, Gunnma prefecture), possibly due to high concentration of sulfate ions originating from volcanic ash (Fig. 5; Supplementary Table S1). Also, the soil $EC_{1:5}$ values and sodium contents in the coastal and semi-coastal habitats of *H. macrophylla* were higher than those of the inland habitats of *H. macrophylla* and the mountainous habitats of *H. serrata*. Basically, soils closer to the sea had higher EC value and sodium contents. However, some semi-coastal areas showed higher EC value and sodium contents than those of the coastal areas (Supplementary Table S1). These findings suggest that soil salinity might be affected by wind direction and how it affects the amount of sea breeze affecting each location (Supplementary Table S2). Another interesting finding was the low soil sodium content at Kashitate in Hachijo-jima, even though the $EC_{1:5}$ of the soil was high. The reason for these contrasting values is likely because the site is located close to an Iwo Pond which contains high concentrations of sulfuric components (Fig. 2), resulting in increased soil $EC_{1:5}$ values and decreased pH. Based on the report by Barrett-Lennard et al. (2008), the soils of the most coastal population habitats could be categorized as having high and severe salinity, which greatly affects growth of most of the plants (Zörb et al. 2019). A similar finding was reported for rice in the Japanese soils affected by the tsunami on 11 March 2011, where subsequent high concentrations of exchangeable Na^+ of ca. 80 mmol/DWkg caused foliar damage on the rice plants (Oi et al. 2013). In our study, the exchangeable Na^+ of the *H. macrophylla* coastal habitats ranged from 13.9 to 62.6 mmol/DWkg, suggesting that *H. macrophylla* is able to adapt to a high salt-stressed environment. Furthermore, in a preliminary experiment we found that *H. macrophylla* could survive under high salinity conditions ($EC_{1:5}$ = ca. 7) for 30 days, whereas *H. serrata* withered and died (data not shown).

In January 2022 (winter season), the defoliation of *H. macrophylla* in Izu-Oshima was still not completed despite it being the winter season, likely because the average winter temperature was higher than on the Honshu mainland. At this time, the Na^+ concentration in the upper leaves of plants from the coastal zones (Nomashi and Okata-shinkai) of Izu-Oshima was significantly higher than those from the semi-coastal zones (Motomachi-akahage and Senzu-fukuju), whereas there was no difference in K^+ concentrations (Fig. 7A). In Okata-shinkai, the leaves of the costal populations accumulated much higher Na^+ concentration (around 1,500 μmol/DWg corresponding to 60 mg/DWg) in comparison with typical concentration; such high Na^+ level usually leads to necrosis and sever damage on the other plants (Liu et al. 2017). Interestingly, in our preliminary experiment using populations from Nomashi, the inner leaves of the winter buds accumulated lower Na^+ and higher K^+ compared with of the remaining mature leaves (Fig. 7A). The maintenance of the K^+/Na^+ homeostasis is known to be one of mechanisms of salinity tolerance (Assaha et al. 2017). Thus, these results suggest that *H. macrophylla* regulates allocation of

toxic Na^+ in plant bodies while maintaining K^+, which enables them to survive the winter season in salt-stressed habitats.

The coastal populations from Nakanogo on Hachijo-jima had higher Na^+ concentrations than the other populations on the island, as well as Okata-shinkai on Izu-Oshima (Fig. 7B). The stem height of both coastal populations of Okata-shinkai and Nakanogo ranged from 5 to 10 cm (data not shown). On the other hand, all the other samples in this study consistently showed around 100 cm of the stem height. Thus, Na^+ accumulation in the coastal populations might depend on morphological traits such as leaf number and stem length. Even though the soils in Mitsune (coast) and Sueyoshi (semi-coast) of Hachijo-jima were affected by salt stress (Supplementary Table S1), the native individuals growing there displayed little difference in Na^+ concentration in their leaves compared to the inland population of Kashitate of Hachijo-jima where is subjected to lower Na^+ contents in the soil (Fig. 7B insert). Additionally, there was no difference in leaf K^+ concentration between the salt-stressed coastal and non-salt-stressed inland plants on Hachijo-jima. In our analysis for Na^+ concentrations, only upper leaves (young leaves) were used. Therefore, large plant size of *H. macrophylla* plants growing in saline soils might suppress the accumulation of the harmful Na^+ in the young leaves being reproductive organs from the lower tissues. In contrast, when plant size is small, the leaves of *H. macrophylla* might resist the toxicity risk of high Na^+ accumulation by the other mechanisms (e.g., accumulation of osmolytes such as proline and glycinebetaine and antioxidation shown in a review by Munns and Tester 2008), but not restrict K^+ uptake. Therefore, future studies should assess Na^+ allocation in various plant tissues and osmolyte biosynthesis to clarify mechanism of adaptation to saline environments.

Conclusions

While the salinity tolerance of ornamental hydrangeas developed from wild plants of *H. macrophylla* has been assessed, there was still little knowledge about the native habitats salt stress adaptation of wild *H. macrophylla*, even though it is an important genetic resource for both breeding and landscape. Thus, in this study, we surveyed the habitats and distribution of *H. macrophylla* endemic to and assessed their adaptation to coastal environments affected by salt stress. *H. macrophylla* tended to prefer relatively humid in rooted zone and was able to grow in oligotrophic environments such as rocky surfaces and volcanic soils mostly comprised of scoria. Additionally, cation analysis of soils and leaves showed that *H. macrophylla* was tolerant to severe salt stress. This report provides foundational data for future studies on ecology and abiotic stress in wild *H. macrophylla*.

Declarations

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Figures

Fig. 1

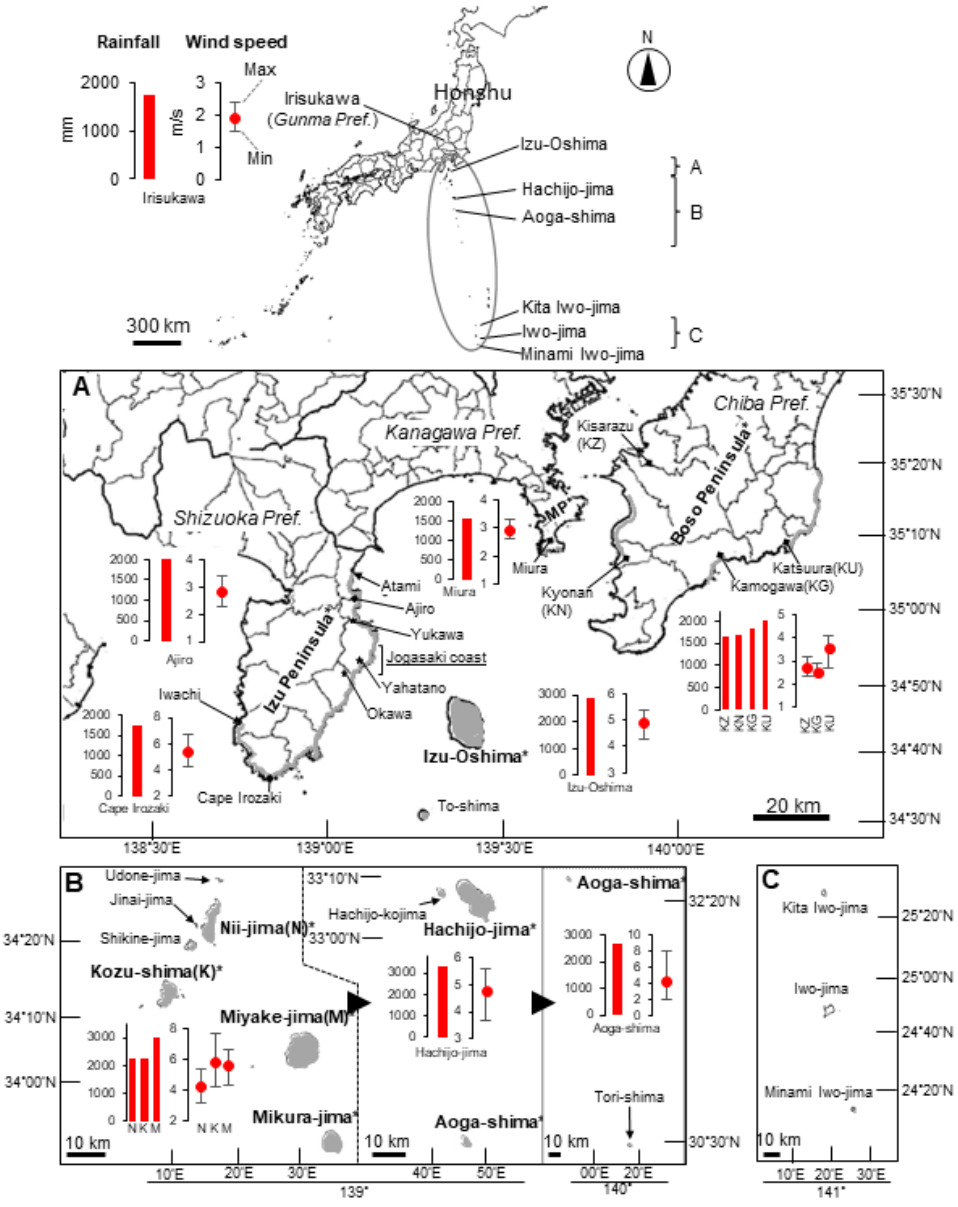

Figure 1

Distribution of *H. macrophylla* endemic to Japan

The enlarged maps show distributions in the three peninsulas of Honshu and Izu-Oshima and To-shima (A), the Izu islands other than Izu-Oshima and To-shima (B), and the Bonin islands (C). Grey colored areas show *H. macrophylla*habitats. Bars and dots indicate mean annual rainfall (mm) and wind speed (m/s), respectively, with the upper and bottom of vertical lines showing maximum and minimum annual wind speed. Asterisks (*) indicate the peninsulas or islands surveyed in this study. In map A, stars (□) show inland distribution. The triangles on map B indicate more southerly islands. MP, Miura Peninsula; KZ, Kisarazu; KN, Kyonan; KG, Kamogawa; KU, Katsuura; N, Nii-jima; K, Kozu-shima; M, Miyake-jima.

Fig. 2

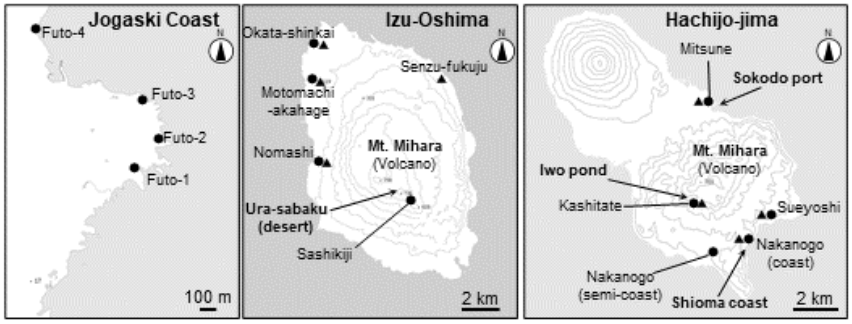

Figure 2

The sampling sites of soils and leaves in Jogasaki coast, Izu-Oshima, and Hachijo-jima.

Circles and triangles indicate sampling sites of soils and leaves, respectively.

Fig. 3

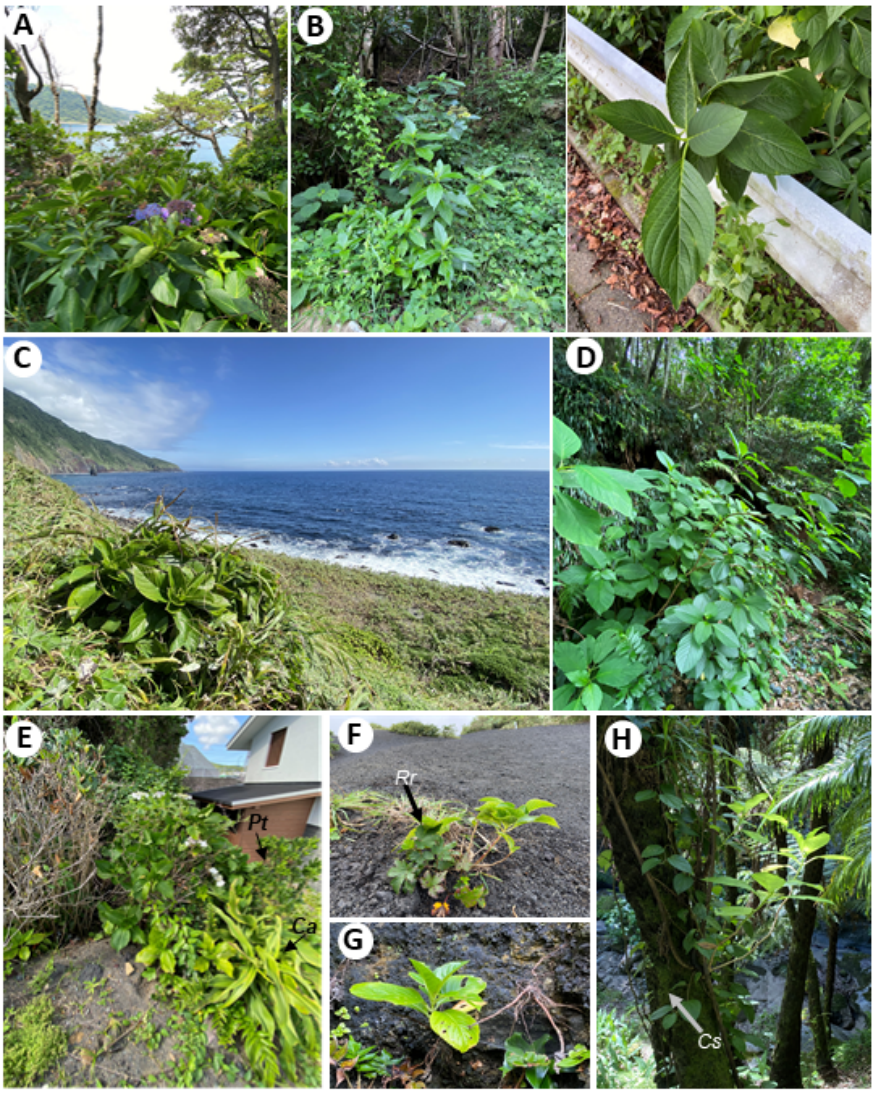

Figure 3

Photographs of native populations and habitats of *H. macrophylla.*

(A) Jogasaki coast on Izu Peninsula.

(B) Okawa (left) and Yahatano (right) on Izu Peninsula.

(C) Close to sea on west coast of Izu-Oshima.

(D) Plants growing among mountain vegetation in Kashitate on Hachijo-jima.

(E) Coastal population at Mitsune on Hachijo-jima, growing beside *Pittosporum tobira* (Pt) and *Crinum asiaticum* var. *japonicum* (Ca).

(F) Individual growing beside *Rubus ribisoideus* (Rr) in Ura-sabaku (desert) on Izu-Oshima.

(G) A seedling growing on a constantly wet rocky cliff on Shioma coast of Hachijo-jima.

(H) Seedlings attached to stem surface of *Cyatbea spinulosa* (Cs) at Nakanogo on Izu-Oshima

Fig. 4

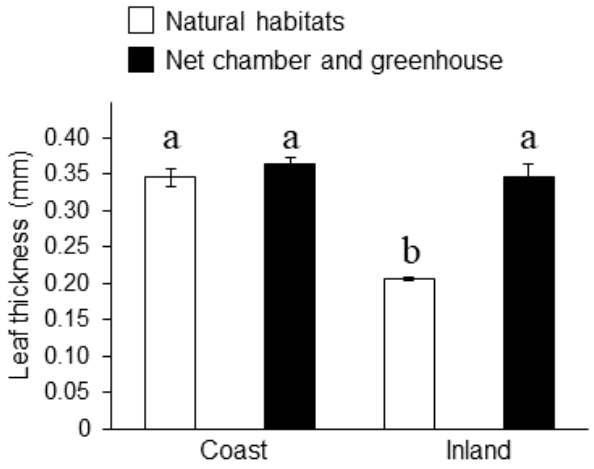

Figure 4

Plasticity in leaf thickness of *H. macrophylla* grown *in situ* and *ex situ*.

Leaf thickness of two populations growing in native habitat or in net chamber and green house at Meiji University. Different alphabetical letters indicate significant difference by Tukey's multiple comparison test ($P < 0.01$, n = 3).

Fig. 5

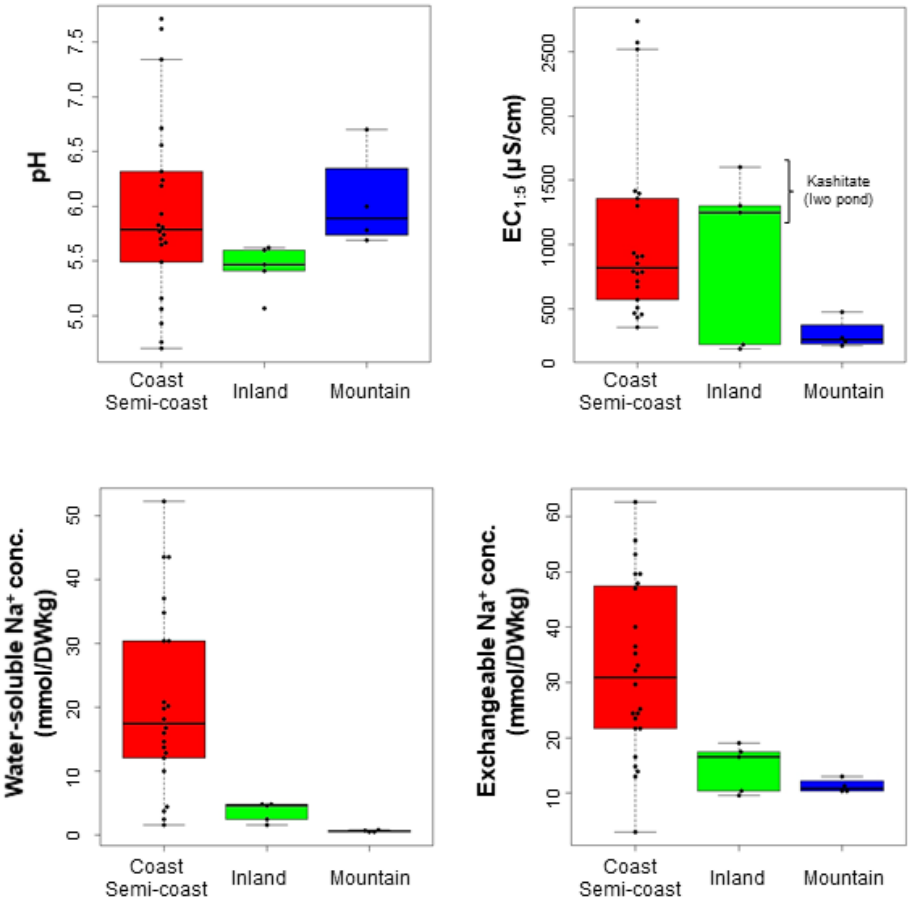

Figure 5

pH, $EC_{1:5}$ and sodium contents in soils collected from native habitats of *H. macrophylla* and *H. serrata*.

The soil sampling sites represent coastal/semi-coastal (n=22) and inland (n = 5) habitats of *H. macrophylla*. The four soil samples collected from the habitat of *H. serrata* were used as control (Mountain).

Fig. 6

Figure 6

Morphological adaptation to salt stress in a coastal habitat.

Photographed on Izu-Oshima on September 5, 2022. Arrows show nodes caused by defoliation.

Fig. 7

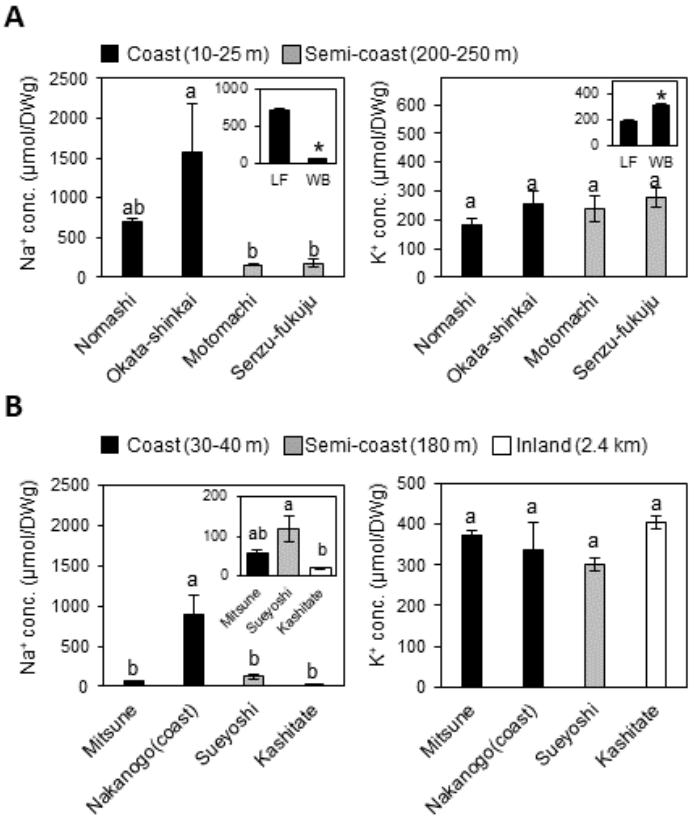

Figure 7

Na^+ and K^+ concentrations in the upper leaves of coastal, semi-coastal and inland plants on Izu-Oshima (A) and Hachijo-jima (B).

The numbers in the parenthesis show the distance from the sea. The different alphabetical letters show significant differences by Tukey's multiple comparison test ($P < 0.05$, n =3). Inserts in graph A show data

from remaining leaves (LF) and winter buds (WB) in Nomashi. Asterisks indicate significant differences by student's *t*-test ($P < 0.05$, n =3). The insert in graph B shows enlarged data excluding Nakanogo (coast).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable230602.pptx