Original article

Preliminary evaluation of ecological and agricultural characteristics of vetiver *(Chrysopogon zizanioides)* maintained in terraced arable fields along the Uwa Sea region, southwestern Japan

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Abstract

The objective of this study was to determine the history, usage, and presence of the seeds of vetiver (*Chrysopogon zizanioides* (L.) Roberty) sporadically distributed in the temperate coastal area of the Uwa Sea region, southwestern Japan. The species identity was confirmed based on both molecular identification using DNA barcoding and analysis of spikelet morphology. According to local farmers, vetiver was initially introduced in the 1950s to protect the juvenile orange tree seedlings from sea wind and the terraced fields from erosion. Vetiver plants were mostly found on terraced farms and no individuals had escaped. Mown vetiver straw has been used extensively as mulch for orange trees and subsistence vegetable culture. Until recently, vetiver plants propagated by division have been transplanted in this region. Eighteen vetiver inflorescences collected from eight clones in three localities in 2017 were found to bear no seeds. These results suggest that these local vetiver accessions are probably non-weedy and can grow well in the temperate coastal areas of Japan.

Key words: folk classification, hedge, mulching, terraced fields, windbreak

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Introduction

Hedge plants are managed to protect farmland boundaries and crops from wind damage. However, they are also used to conserve local flora and fauna, serve as food sources and materials for making tools, and also shape countryside landscapes (Forman and Baudry, 1984; Burel, 1996). Descriptions and distributions of hedge plants are well documented in European agricultural landscapes. Nevertheless, there are comparatively sparse records of hedge plants from other regions (Baudry et al., 2000). The identification of agriculturally and ecologically suitable hedge plants may potentially help improve sustainable development and rural landscape management.

We found a sporadically distributed alien grass species, vetiver (*Chrysopogon zizanioides* (L.) Roberty), among the hedges on certain terraced

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farmlands along the Uwa Sea region. Vetiver is thought to be native to the Indian subcontinent (Lavania, 2000). It was widely introduced throughout Asia, Africa, Central America, the Caribbean, and the Mediterranean (Grimshaw, 1993; Dudai et al., 2006). Vetiver has been globally and widely utilized as a source of oil that is extracted from its root, protection of soils, and providing materials for manufacturing various artifacts (Grimshaw and Helfer, 1995). In Japan, some universities and experimental institutes maintained vetiver imported from the island of Java in Indonesia during the pre-World War II period (Nihon-Kouteisho-Kyoukai, 1970). According to Nihon-Kouryou-Kyoukai (2009), since around 1948, commercial cultivation of this grass for oil production began in the warmer areas of Japan, such as in Amami Oshima and Tanegashima in Kagoshima Prefecture. However, cultivation in these areas declined owing to the technical difficulty in distilling oil and rising wages in the late 1960s. In the early 1950s, the chemical properties of essential oils in the root extracts of vetiver were analyzed on the Boso Peninsula, Chiba Prefecture, Japan (Kawana et al., 1954), and the growth of the vetiver root was examined in Tanegashima in Kagoshima Prefecture (Ouchiyama and Matsushita, 1955). On the Izu Peninsula, Shizuoka Prefecture in the 1950s, vetiver was used as a hedge plant to protect marguerite (*Argyranthemum frutescens* (L. Sch.Bip.)) planted on terraced farmlands from strong wintertime sea winds (Matsui, 1968). Vetiver was evaluated as a candidate pasture grass species for plantation in the Tokai region (Ishida et al., 1967) and the Kyushu region (Oyama and Nakagawa, 1981). In the 1970s, the Ministry of Agriculture and Forestry of Japan (MAF) recognized vetiver as a useful tall grass and recommended its cultivation to prevent soil erosion and improve public land (MAF, 1977). In the recent decade, Okinawa Prefectural government (Sakata et al., 2010) and some non-profitable organizations such as Okinawa Green Network (http://okinawagreen.net/greenbelt/index.html) and Useful Plants Spread Society (http://www.uf-plants.com/katsudou.html) have been promoting the use of this plant for soil protection purposes in several places in Japan. However, the background of vetiver distribution along the Uwa Sea region remains unknown. In addition, the ecological and agricultural characteristics of vetiver usage in Japan have not yet been well documented.

Farming terraces were traditional coastal landscapes along the Uwa Sea region. Crops most commonly raised earlier in the history of terraced farming included sweet potato, wheat, and barley. For centuries, they were mainly cultivated as subsistence agriculture (Miyamoto, 2006). Since the 1950s, however, they have largely been replaced by commercial orange plantations (Ehime Prefecture, 2014). To protect oranges from sea winds, trees like *Cryptomeria japonica* (L.f.) D. Don and *Podocarpus macrophyllus* (Thunb.) Sweet var. *spontaneus* H.Ohba et. S.Akiyama were planted as hedges. Native grass species such as *Arundo donax* L. have also been trimmed and maintained as hedges. However, there are no records of the use of alien grasses such as vetiver as hedges in this region.

The objective of this study was to determine the introduction history, past and present usage, and weediness of vetiver grass in the Uwa Sea region. Information sources included grass species identification, field observations of grass distribution, interviews with local farmers, and examination of the sessile spikelets for the presence of seeds.

Materials and Methods

Species identification

The grass species was identified based on DNA barcoding and analysis of spikelet morphology. The plant samples were collected from Mizugaura village (MZ1) and the southern and northern parts of Shuki village (SK1 and SK2, respectively) on 2nd December 2017. Voucher specimens of the plants were deposited in the Ehime Prefectural Science Museum; the accession number was 16861 for MZ1, 16860 for SK1, and 16859 for SK2.

1) Molecular identification

Leaf samples were used in molecular identification. Approximately 10 mg DW leaf tissue from each sample was put in a microtube along with two stainless steel beads (diameter 5 mm). The microtubes were placed in an aluminum block pre-chilled to -80°C. The block was shaken for 2 min at maximum speed with a Shake Master (Bio Medical Science, Tokyo, Japan). DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer’s protocol. DNA barcoding analysis was conducted using the *trnL* intron and *trnL-trnF* intergenic spacer of chloroplast DNA (*trnL-trnF*). For PCR amplification and sequencing, the primer combination was trnL-c (forward): 5′-CGAAATCGGTAGACGCTACG-3′ and trnF-f (reverse): 5′-ATTTGAACTGGTGACACGAG-3′ (Taberlet et al., 1991). The amplification reaction mixture consisted of 0.08 μL of 5 U μL^{-1} Ex Taq polymerase (TaKaRa Bio, Shiga, Japan), 1.6 μL Mg^{2+}-free Ex Taq buffer, 1.3 μL of 25 mM $MgCl_2$, 1.3 μL dNTP mixture (2.5 mM each), 0.5 μL DNA template, 0.5 μL each forward and reverse primers (10 μM), and up to 16.6 μL purified water. The PCR cycles consisted of an initial denaturation step for 4 min at 94 °C, 35 denaturation cycles (30 s at 94 °C), annealing (40 s at 52 °C), extension (1 min at 72 °C), and a final extension at 72 °C for 10 min. The PCR products were checked by electrophoresis on 2% (w/v) agarose gel, treated with ExoSAP-IT (USB Corp., Cleveland, OH, USA), and directly sequenced in both directions in an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) using the same primers as those for PCR. The sequences were deposited in the DNA Data Bank of Japan (DDBJ accession numbers LC386313 to LC386315). The sequences showing the highest similarities with those of our samples were retrieved from the GenBank/NCBI database (National Center for Biotechnology Information, Bethesda, MD, USA; 2018) using the Basic Local Alignment Search Tool (hereafter, BLAST; Altschul et al., 1990).

2) Morphology

Based on the results of molecular identification, by referring to the morphological information in GrassBase (Clayton et al., 2006 onwards), spikelet morphology of our samples was compared with those of the candidate species in *Chrysopogon* clustered within a monophyletic group.

Study area

The focal grass species were explored along the coastal and lowland inland areas of the southwestern part of the Shiko region. Nevertheless, the grass was actually found in the coastal areas of Seiyo City and Uwajima City (Fig. 1). In both localities, the grass individuals were found mostly on terraced farms.

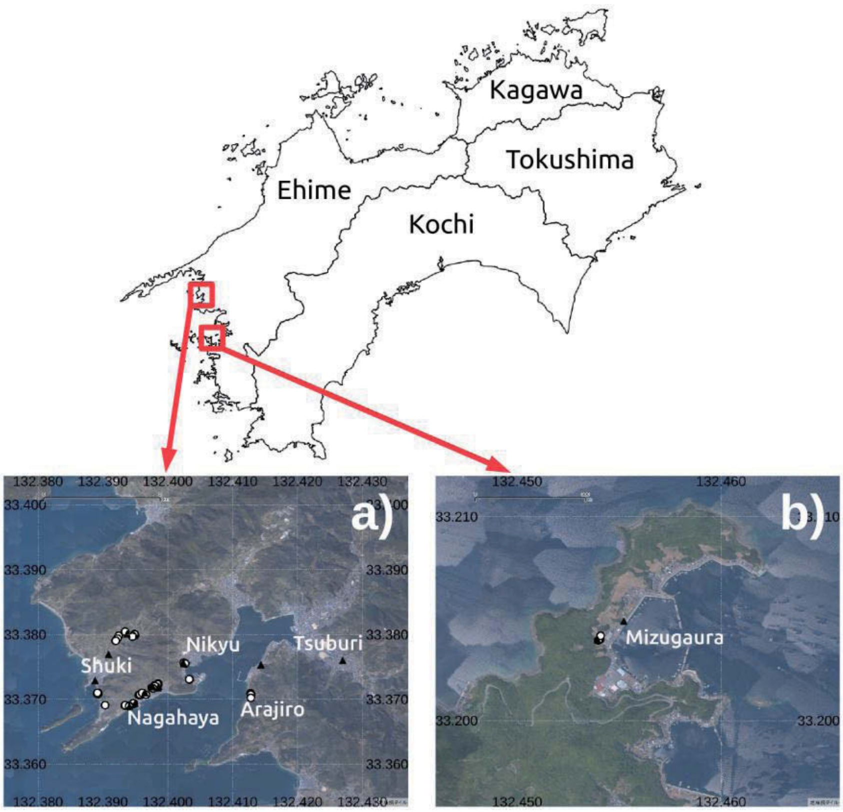

Fig. 1. Maps of study site. a) Mikame, Seiyo City, and b) Mizugaura, Uwajima City. Local district names on each map are shown in bold letters. Open circles and closed triangles indicate the focal grass and the interviewee locations, respectively.

No individuals had escaped. The map of Shikoku island and aerial photographs were obtained from the Geospatial Information Authority of Japan (2018). Maps of the study sites were created with Quantum GIS v. 2.18 (Quantum GIS Development Team, 2018). The study sites are located in a temperate zone and contain coastal terraced farmlands. Data recorded at the Uwajima Weather Station from 2001 to 2015 (Japan Meteorological Agency, 2018) indicated that the mean monthly temperature was 17.0 °C and the mean annual rainfall was 1,739 mm.

Verification of the presence of seeds

Eighteen inflorescences were collected from the three locations. Six each were obtained from one clone in Mizugaura village, from three clones in the southern part of Shuki village, and from two clones in the northern part of Shuki village. Thirty sessile spikelets per inflorescence were randomly chosen for stereomicroscopic verification of the presence of seeds.

Interview

When a grass species was detected in the study area, local crop managers or neighboring farmers familiar with the plant were interviewed. After obtaining verbal consent, fifteen individuals or pairs of interviewees (13 from Seiyo City and two from Mizugaura, Uwajima City) answered predetermined questions in a semi-structured interview. Interviewees were queried about the local names for the grass and its artifacts. The orthography followed the International Phonetic Alphabet and the names were written in bold letters. They were also asked about the reasons for plant use, multiple usages, grass introduction period, acquisition channels, and crop management. Wherever possible, any supplementary information on the grass and its local plant usage was recorded for each informant. Two public officers and a local orchard management professional belonging to the Japanese Agricultural Cooperatives were queried about the documented history of the grass introduction in the region. Nevertheless, they were not able to provide such information.

Plant nomenclature followed The Plant List (2013). Fungal nomenclature followed the GeneBank Project, NARO (National Agriculture and Food Research Organization, Tsukuba, Japan; 2018). Insect nomenclature followed the Global Biodiversity Information Facility (hereafter, GBIF; 2018).

Results and Discussion

Species identification

The DNA barcoding region *trnL-trnF*, which has proven useful in plant identification (Kraaijeveld et al., 2015), was used for species identification. The partial sequences of the *trnL-trnF* region (889 bp) from MZ1, SK1, and SK2 were all identical to each other. In a BLAST search, the sequences of *C. zizanioides* (accession number: KY596158), *C. fulvibarbis* (Trin.) Veldkamp (KY596161), and *C. gryllus* (L) Trin. (GQ867652) showed the maximum similarity (100%, 99.2%, and 98.5% identities, respectively) with those of our samples. Following the results of molecular identification, the spikelet morphology of our samples was compared with those of the three species described in the GrassBase by Clayton et al. (2006 onwards). The presence/absence of geniculate principal lemma awn is a distinct morphological difference among the three candidate species. Principal lemma awn of *C. fulvibarbis* is 10-20 mm long and that of *C. gryllus* is 12-20 mm long. Contrarily, *C. zizanioides* does not have such awn in its lemma, and neither did our samples (Fig. 2). Based on these molecular and morphological information, our samples were identified as *C. zizanioides*.

Absence of seeds

The absence of seeds was confirmed for the 540 sessile spikelets from the 18 inflorescences sampled in the study areas. According to Grimshaw (1993), vetiver grass is not considered invasive in various parts of the world because it is sterile. However, certain accessions have been found to bear viable seeds and spread naturally. In the present study, no escaped vetiver was detected on roadsides or in forest edge environments possibly because the local vetiver accession(s) are sterile. A weed risk assessment for vetiver introduction on the Ogasawara Islands in Japan indicated that introduction of this plant poses a low level of risk to the island environment (Ogawasara Islands Nature Information Center, 2018). The absence of seeds and the lack of escaped individuals in our study regions corroborate the risk assessment for vetiver introduction on the Ogasawara Islands. The only proviso is that the candidate vetiver accessions for plantation on the islands have similar reproductive traits to those of the variety identified at the sites of the present study.

Fig. 2. Photograph of the vetiver spikelets sampled in Mizugaura, Uwajima City.

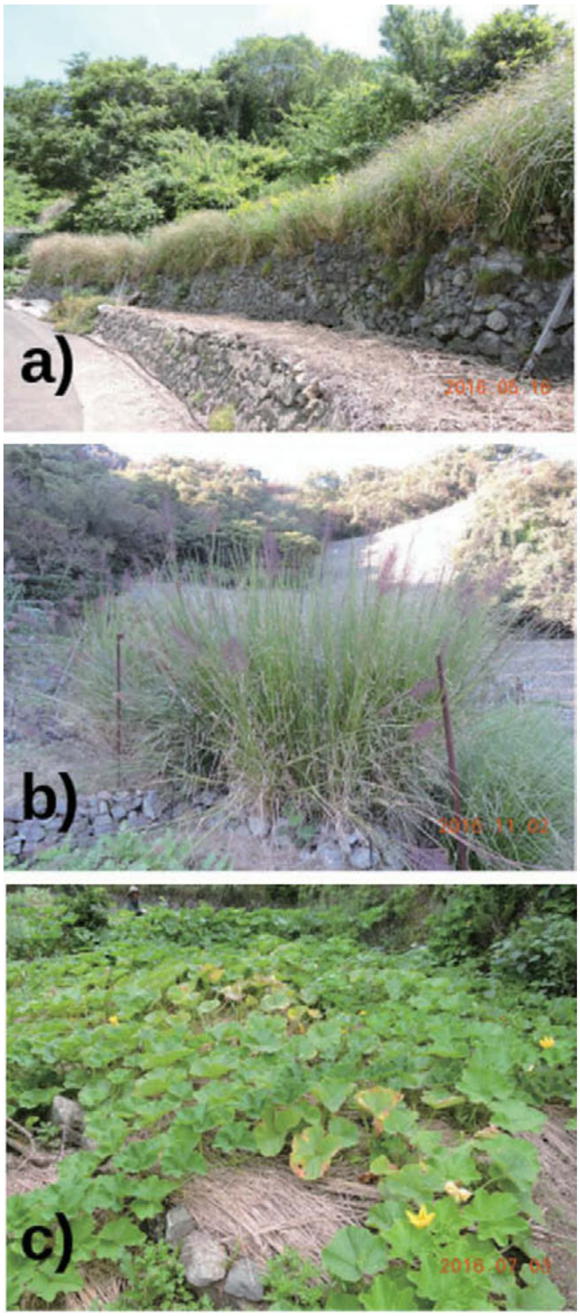

Fig. 3. Vetiver applications in the Uwa Sea region, Ehime Prefecture, Japan. a) A vetiver grass hedge along the edge of a terraced farm in Nagahaya, Seiyo City; b) a flowering vetiver in Mizugaura, Uwajima City; and c) vetiver straw mulch on pumpkins in Shuki village in Seiyo City.

Vetiver introduction history

Vetiver was introduced to the study region primarily during the 1950s and 1960s (n = 8) but also in the 1970s (n = 1), the 1980s (n = 1), the 1990s (n = 1), and between 2010 and 2011 (n = 1). In the initial introduction period, local farmers acquired vetiver seedlings from a branch office of the Japanese Agricultural Cooperative (n = 4 in Seiyo City) and through free sharing of vetiver plants propagated by division among acquaintances (n = 4). Around the same time in other localities in Japan, vetiver was cultivated for perfume oil extraction (Kawana et al., 1954; Nihon-Kouteisho-Kyoukai, 1970; Nihon-Kouryou-Kyoukai, 2009). It was also planted as a windbreak for terraced fields in the Shizuoka Prefecture (Matsui, 1968) and used as a trial candidate species for grassland development (Ishida et al., 1967; Oyama and Nakagawa, 1981). Therefore, vetiver planting occurred at several localities in warm temperate regions of Japan in the middle of the 20th century for such different purposes. In more recent times, this grass in our study regions was propagated by division and shared among acquaintances.

Vetiver planting sites in the present study were located starting from shorelands close to the coastline to ~850 m inland. The plants appeared mostly at the edges of terraced fields (Figs. 1, 3a, and 3b). In coastal landscapes, sea salt sprays onto the vegetation there. However, vetiver distribution and growth habit in the study areas indicate that it is highly resistant to salt damage. This finding corroborates the report of Truong et al. (1991).

None of the informants interviewed was familiar with the common Japanese names for vetiver such as **betɕiba**ː, **betɕiberɯᵝ**, or **betɕiberɯᵝsoɯᵝ**. Two informants in Uwajima City referred to vetiver as **metɕi**ː**ba**. However, this name could be a miscommunication of the common Japanese name **betɕiba**ː. In Mikame, Seiyo City, 11 out of 13 informants called vetiver **kaja**, which is a folk generic name in Japan for various Poaceae species such as *Miscanthus sinensis* Andersson, *Imperata cylindrica* (L.) Raeusch., and certain Cyperaceae species. One informant in Mikame, Seiyo City called vetiver **ragɯᵝrasɯᵝ** which, in the Japanese language, is suggestive of a foreign or alien plant species. This nomenclature may be confused with that for love grass (genus *Eragrostis*). No documentation concerning the introduction of vetiver was available from the prefectural office or the Japanese Agricultural Cooperatives. Moreover, the local farmer in charge of distributing vetiver in Mikame, Seiyo City was deceased. Nevertheless, these folk classification patterns suggest that different people or agencies may be in charge of vetiver seedling distribution at the two localities.

Reasons for vetiver introduction, usage, and management

Vetiver was introduced as a windbreak (n = 9), for protection against soil erosion (n = 2), or both (n = 3). In the southern part of Ehime Prefecture, large areas of the local cropping systems on terraced fields were converted from sweet potato/wheat (or barley) rotation to orange groves between the 1950s and the 1970s (Ehime Prefecture, 2014). According to three informants, vetiver was planted during that time period as a windbreak to protect young orange seedlings from sea spray. Another common usage for vetiver was mown straw for mulching (n = 10) orange and subsistence crops like cucumber, eggplant, melon, squash, and watermelon (Fig. 3c). In Nigeria, vetiver strip and vetiver mulch reduced soil loss and nutrient runoff and improved grain yields in maize production (Babalola et al., 2007). The relative similarity of vetiver usage in different environments suggest that the grass provides multiple benefits as a source of mulch for farmland soil and crop protection. Various traditional artifacts have been made from vetiver in India (Tripathy et al., 2014). In Seiyo City, the woven flat mat locally called **toma** is usually made from the grass *M. sinensis*. However, two informants in the area stated that vetiver might have also been used for this purpose. In the study region, the flat lowland area for paddy field use was very limited. One informant stated that he had to visit relatives at a distant (~10 km) inland region within Uwa City to acquire rice straw for mulching. In the warm temperate coastal areas in Japan, native grass species such as *M. sinensis*, *M. sinensis* subsp. *condensatus* (Hack) T. Koyama, and *M. floridulus* (Labill.) Warb. ex K. Schum. & Lauterb. were planted for use as windbreaks (Miyamoto, 1984; Hokama, 2004; Toyama et al., 2016). *Miscanthus sinensis* was commonly distributed throughout the areas investigated in the present study. However, two informants stated that vetiver straw was easier to handle than that of *M. sinensis* because the leaf margins of the former were not as sharp as those of the latter.

Vetiver management practices varied among informants. These included no management at all (n = 3), no systematic management (n = 1), irregular mowing as needed (n = 2), annual mowing (n = 2), and semiannual mowing (n = 2). Therefore, there is apparently no strict mowing practice to maintain vetiver in coastal environments. Vetiver growth may become dense but it does not vegetatively propagate with creeping rhizomes toward surrounding spaces as do other grasses like *M. sinensis*. This trait seems to facilitate the extensive management of vetiver.

Vetiver as a possible host for certain crop pests

While vetiver has multiple uses, certain studies reported that it may also be a host plant for crop pests. In a laboratory experiment, the bacterial orange pathogen *Xanthomonas citri* subsp. *citri* (ex Hasse) Gabriel, Kingsley, Hunter & Gottwald survived at low concentrations for 200-300 d on vetiver straw as well as lawn grass and rice straw (Goto et al., 1975). Nevertheless, this risk must be evaluated in field conditions before concluding that vetiver is, in fact, the actual disease vector. Vetiver is a host plant for the insect pest *Heptophylla picea* Motschulsky, which is known to damage many plants including tea (*Camellia sinensis* (L.) Kuntze) (Otaishi, 1988). Tea plants are often cultivated on a small, non-commercial scale. They were commonly observed in the gardens of the study areas. Therefore, it is expected that the impact of vetiver on tea plants would be minimal even if the insect pest appeared.

Conclusions

Although about half a century has passed since the initial introduction of vetiver to the study region (Uwa Sea), some farmers in both localities expressed their appreciation for its utility. Vetiver may not always be suitable for windbreak plantings because of its limited height; it may not be able to protect tall crops like adult orange trees. However, it is beneficial for soil protection and as a vegetable crop windbreak. Furthermore, its solid stems can be used for mulching various crops. Vetiver can be readily maintained by extensive mowing management and can benefit farmers. The present study preliminarily confirmed the absence of seeds in the sessile spikelets from the three locations. Therefore, the local vetiver accessions are probably sterile. Research goals in future studies include verification of the sterility in local vetiver populations and the assessment of the risk that vetiver could escape. In addition, the contribution of vetiver to sustainable agriculture in warm temperate coastal environments should be evaluated.

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Competing interests

The authors declare that they have no competing interests.

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宇和海沿岸の段畑に維持されてきた外来イネ科草本ベチベルソウの生態および農業上の特性に関する予備評価

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本研究では温暖な西南日本に位置する宇和海沿岸に散見されたベチベルソウの来歴、利用例、種子の結実の有無の評価を目的とした。種の同定はDNAバーコーディングを用いた分子同定および小穂の形態観察に基づいて行った。地域の農家によれば、ベチベルソウははじめに1950年代頃に蜜柑の苗木を潮風から保護するため、あるいは段畑の土壌流亡防止のために導入されていた。ベチベルソウはそのほとんどが段畑内に観察され、逸出個体は見つからなかった。刈り取ったベチベルソウの葉は今日まで蜜柑や自給的に栽培されている野菜のマルチとして利用されていた。また最近まで無性生殖により増殖したベチベルソウの苗の植栽が継続されていた。2017年に3ヶ所に分布していた8クローンから採取された18の花序の観察では種子は見つからなかった。これら結果は、対象地域のベチベルソウの系統にはおそらく雑草性が無く、国内の沿岸温帯地域で良好に生育できることを示唆している。

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