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Decreasing of Population Size of *Imperata cylindrica* Mangrove Ecotype and Sea-Level Rising

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1. Introduction

A very recent on-going trend worldwide is that sea level is found rising up due to global warming (Cazenave and Llovel, 2010). With one to two degrees temperature elevated, the polar iceberg will melt which eventually will lead to significant sea-level rise. There is increasing interest in knowing how mangrove population changes under sea-level rising. Mangrove forest (mangrove) is usually termed "kidney of the earth". The morphology and anatomy of these plants have unique structure, such as breathing roots, knee roots. Viviparous seedlings of the Rhizophoraceae plants further drift with the sea which results in implantation and reproduction. The origin of the name mangrove is derived from plants of a kind of mangrove tree *Rhizophora mucronata*. The plant's timber, tree trunks, branches, flowers are red. These trees grow in tropical and subtropical regions of rivers and coastal salt marshes, evergreen shrub or tree forest.

According to the literature, mangrove forests are mainly distributed in the western coastal estuary in Taiwan (Hsueh and Lee, 2000). Taiwan's largest and most well-known mangrove protected areas are in Danshui district, including Chuwei mangrove conservation area. These areas are along the Danshui River. The main object of the protected areas is *Kandelia* mangrove forests. *Kandelia candel* (L.) Druce is grouped into Rhizophoraceae *Kandelia* genera. Kandelia is the dominant species and turned out to be a precious national treasure along the Danshui River in Taipei, Taiwan. In addition to *Kandelia*, there are a wide range of plants associated with its surrounding area. These plants have also evolved special morphological and physiological characteristics to adapt to flooding and high salinity environment. These halophytes include *Phragmites austrakis* (reed), *Sporobolus virginicus* (salt sage), and *Imperata cylindrica* (cogon grass). In 1982 Lue carried out an ecological survey of Chuwei mangrove forest, and found the population of *Imperata cylindrica* (Lue, 1982).

Imperata cylindrica, a perennial herb, is a C4-type plant. It has 10 pairs of chromosomes. A single *Imperata* individual can generate many seeds. Seed dispersal can be assisted by wind. *Imperata* can also use their underground rhizome for asexual reproduction. It is adapted to a

wide range of environmental factors and confers a high degree of stress tolerance. The underground rhizome is a commonly used medicinal herb. There are about 10 species of *Imperata* worldwide. In Taiwan there is only one genus one species, namely *Imperata cylindrica* (L.) Beauv. var. *major* (Nees) Hubb. (Hsu, 1975). Cheng & Chou (1997a) examined the leaves of *I. cylindrica* from Chuwei using scanning electron microscope (SEM) and found that Chuwei population differs from others in anatomy and morphology. The stele was empty instead of solid. The lower stem was surfaced with white wax instead of trichomes. In addition, polymorphism among populations was also analyzed by rapid amplify polymorphic DNA (RAPD) (Cheng & Chou, 1997b) and restriction fragment length polymorphism (RFLP) (Chou & Tsai, 1999) on ribosomal DNA (rDNA) (Chiang *et al*., 1998; Tsai & Chou, 1999). In 2006 Chang found that Chuwei population is salt and flood tolerant (Chang & Chou, 2006). In 2008 Chang utilized proteomics approach and identified differentially expressed protein among Chuwei, Sarlun and Neihu Imperata populations (Chang, 2008). All these results revealed that Chuwei population was found to be a distinct ecotype.

Under salt pressure, plants accumulated various organic compounds *i.e.* proline, glycinebetaine, choline, glycerol, and sorbitol as compatible solutes in their bodies. Barley (Stewart & Michelle, 1983), tobacco (Binzel *et al*., 1987), eggplant (Jain *et al*., 1987), and spinach (Coughlan & Wyn Jones, 1980) accumulated large amounts of proline in response to salt treatment. The amount of proline increased with increased salinity. In addition, the expression of proline was regulated by abscisic acid (ABA) (Stewart, 1980; Stewart & Voeberg, 1985). Proline played diverse roles in stress physiology. Stewart & Lee (1974) pointed out that the accumulation of proline in plants correlated its tolerance to salt stress. They also indicated that high level proline would protect many N metabolism-related enzymes from harm. Moreover, large amount of proline would inhibit ACC from converting to ethylene so that plant would not hurt by ethylene (Chrominski *et al*., 1988; Chrominski *et al*., 1989). Proline could also be osmo-protectant which functions to maintain osmotic potential balance (Jain, 1987). It is suggested that proline in *I. cylindrica* from Chuwei may function as an osmo-regulator against salt stress.

Mangrove swamp environment is very unique. It is not only a salty land but is also subjected to periodic flooding which is very detrimental to plant growth. It has been discussed that sea-level rise may impact the survival of mangrove forests (Feller *et al*., 2010). The rises of sea water may impact the survival of mangrove trees in the world. We suspect that the rise of sea water may be one of the causes of the population size decrease of Chuwei mangrove population of *Imperata*. A field work survey discovered that the population size of Chuwei ecotype is decreasing. The proline content of Chuwei population in the field before and after flooding was measured. In present study, we tried to investigate the relationship between the decrease of population size of *Imperata* Chuwei ecotype and proline content changes. We hypothesized that the dynamics of proline content may be related to the population size decrease. The study of how flooding affects proline accumulation may elucidate the possible impact of sea-level rise using Imperata mangrove population as an example.

2. Materials and methods

2.1 Sampling sites and plant materials

Imperata cylindrica (L.) Beauv. var. *major* (Nees) Hubb, Cogon grass, was used as plant materials. Chuwei mangrove salt marsh wetland (Hwang & Chen, 1995) where periodical

flooding pressure threatens plant survival was chosen as a sampling site (Figure 1, Figure 2). Sarlun sandy beach was chosen as a control site (Figure 1, Figure 3) (Figure 1, 2, and 3 were adapted and modified as previously described (Chang, 1996)). These two sampling sites are close to each other in Danshui area, Taipei as shown in Figure 1. Plant leaves from Chuwei were harvested between July and October in 1995 and 2011 for proline content assay. Plant samples were collected on spring-tide days (high tide and flooding; Jul 14, Aug 13, Sep 11, Sep 28 and Oct 12 in 1995; Feb 20 in 2011). During harvesting, each leaf sample was excised by sterilized scissors and stored in zip-block in ice bucket with dry ice to keep it fresh and brought back to lab immediately for study the same day. Plant leaves harvested from each site were assay for water proline. Plants rhizomes collected from the field were washed by sterilized water and cultured in pots (60× 20 ×20 cm^3) in greenhouse for two weeks, then transplanted to Kimura's culture solution (Chang & Chou, 2006) to grow plantlet, the vegetative shoot. The culture solution was aerated with air pump without break. The culture solution was changed every week. These plantlets were used for differential display assay.

2.2 Hypotheses to be tested

We have two hypotheses for the interpretation of possible reasons for the population size decrease of Chuwei ecotype. The first hypothesis is a synecology hypothesis. This hypothesis address that the overpopulated *Kandelia* may threaten the survival of Chuwei Imperata. The second hypothesis is an autoecology hypothesis. This hypothesis address the local flooding may affect the survival of Chuwei Imperata. We hypothesized that Chuwei ecotype may respond to local flooding in a manner different from *Kandelia*. We suspected that local flooding may impact the survival of Chuwei ecotype even though it is flooding tolerant (Chang and Chou, 2006).

2.3 Proline content measurement

L-proline content was measured by the method as previously described (Bates *et al*., 1973). Half a gram of plant fresh leaf tissues were chopped into pieces and quick-frozen in liquid nitrogen in 5 g sea sand and 5mL extraction buffer containing 3 % (w/v) 5-sulfosalicylic acid for grinding and homogenization. The homogenates were centrifugated at 538×g (Sigma, Model 2K15, Taiwan) for 10 minutes. The supernatant was obtained as proline crude extract. The reaction mixture contained 2 ml acid-ninhydrin solution, 2ml proline crude extract. Acid-ninhydrin solution contained a mixture of 0.14 M ninhydrin, 60 % (v/v) acetic acid, 2.4 M phosphatic acid and 2 ml acetic acid. The reaction took place at 100℃ for 1 hour. Samples were then put into -20℃ refrigerator to stop the reaction immediately. Four ml methane was mixed with samples and vortex viguously. The solution was divided into two layers, the upper methane layer and the lower water layer. Three ml upper ethane layer solution was placed in a cuvette. Proline content was analyzed spectrophotometrically at wavelength 520nm using spectrophotometer (Beckman, Model DU-50, Taiwan). Ten replicas were included for each data set. Data were analyzed by Student□s pairwise t-test in SPSS statistical package.

2.4 RNA extraction

RNA extraction was conducted by use of pine tree method as previously described (Chang *et al*., 1993). Leaves of Sarlun and Chuwei population of *Imperata cylindrica* were ground in liquid nitrogen using mortar and pestle. About 2 grams of leaf powder were added into 15 mL extraction buffer containing 100 mM Tris-HCl pH 8.0, 25 mM EDTA, 2 M NaCl, 0.5 g/L

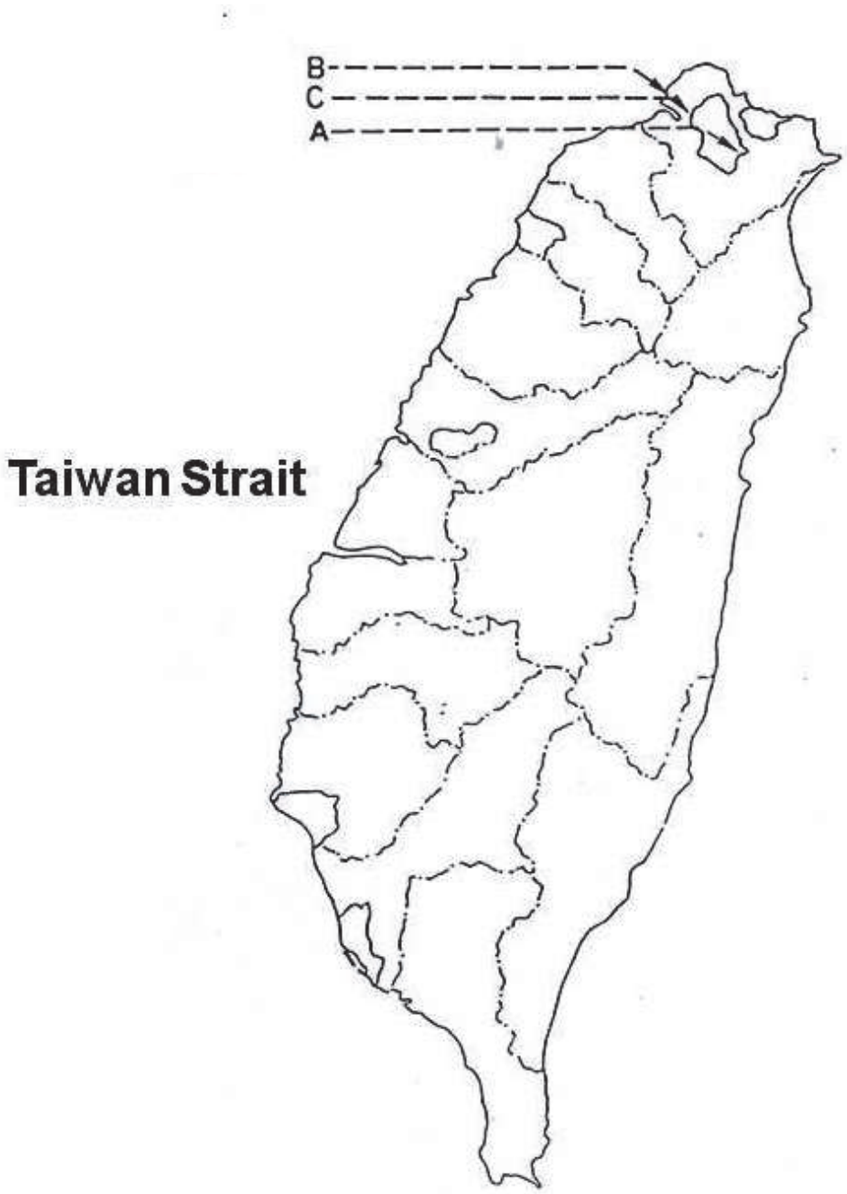

Fig. 1. Sampling sites of *Imperata cylindrica* in Danshui area, Taipei, Taiwan. *Imperata cylindrica* sampling sites were labeled as arrows indicated. A: Neihu; B: Sarlun; C: Chuwei.

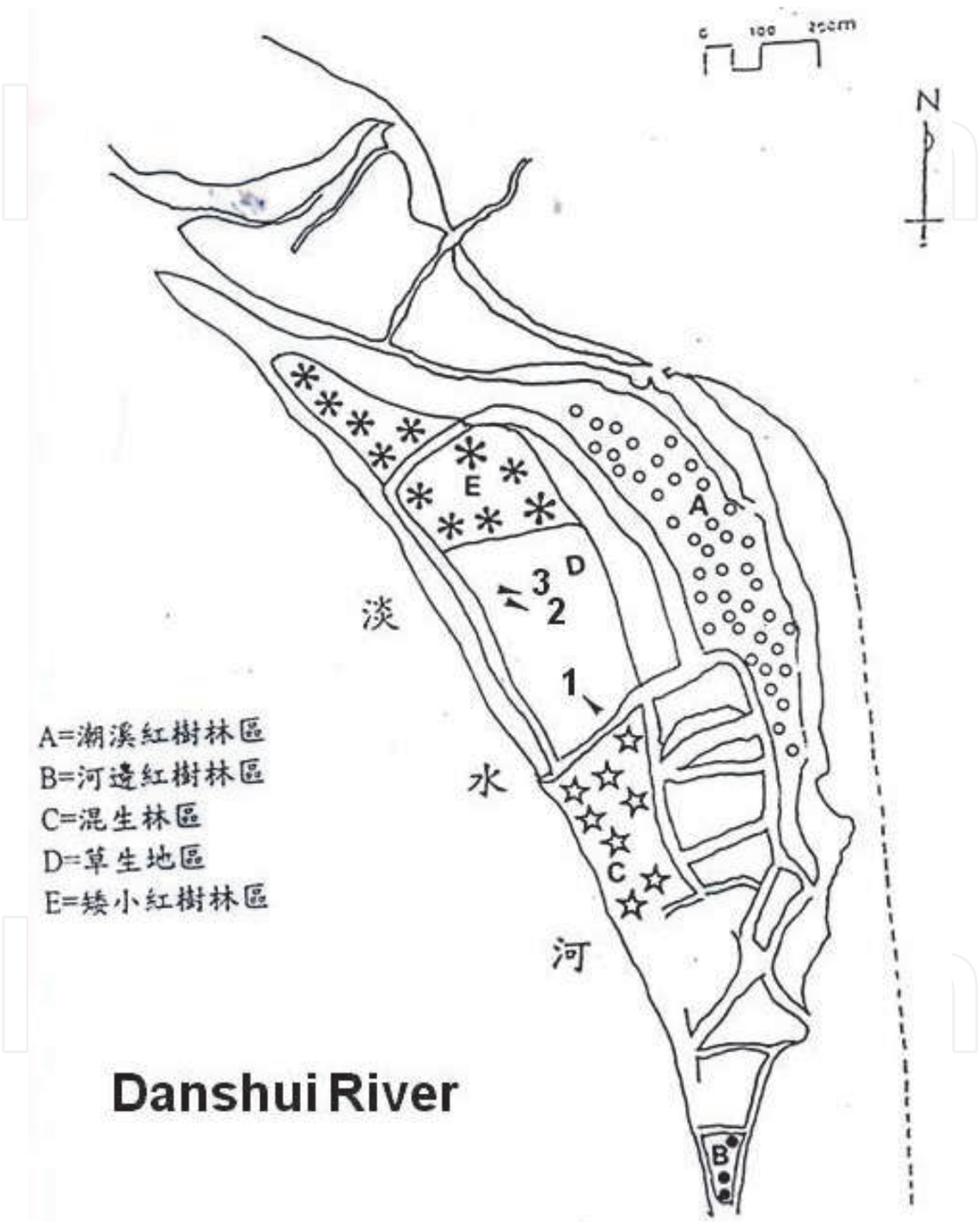

Fig. 2. Sampling sites of *Imperata cylindrica* in Chuwei, Taipei, Taiwan. *Imperata cylindrica* is populated in area D. In area D, sampling site 1, 2, and 3 were labeled as arrows indicated. Site 2 and 3 were lost just very recently for unknown reasons.

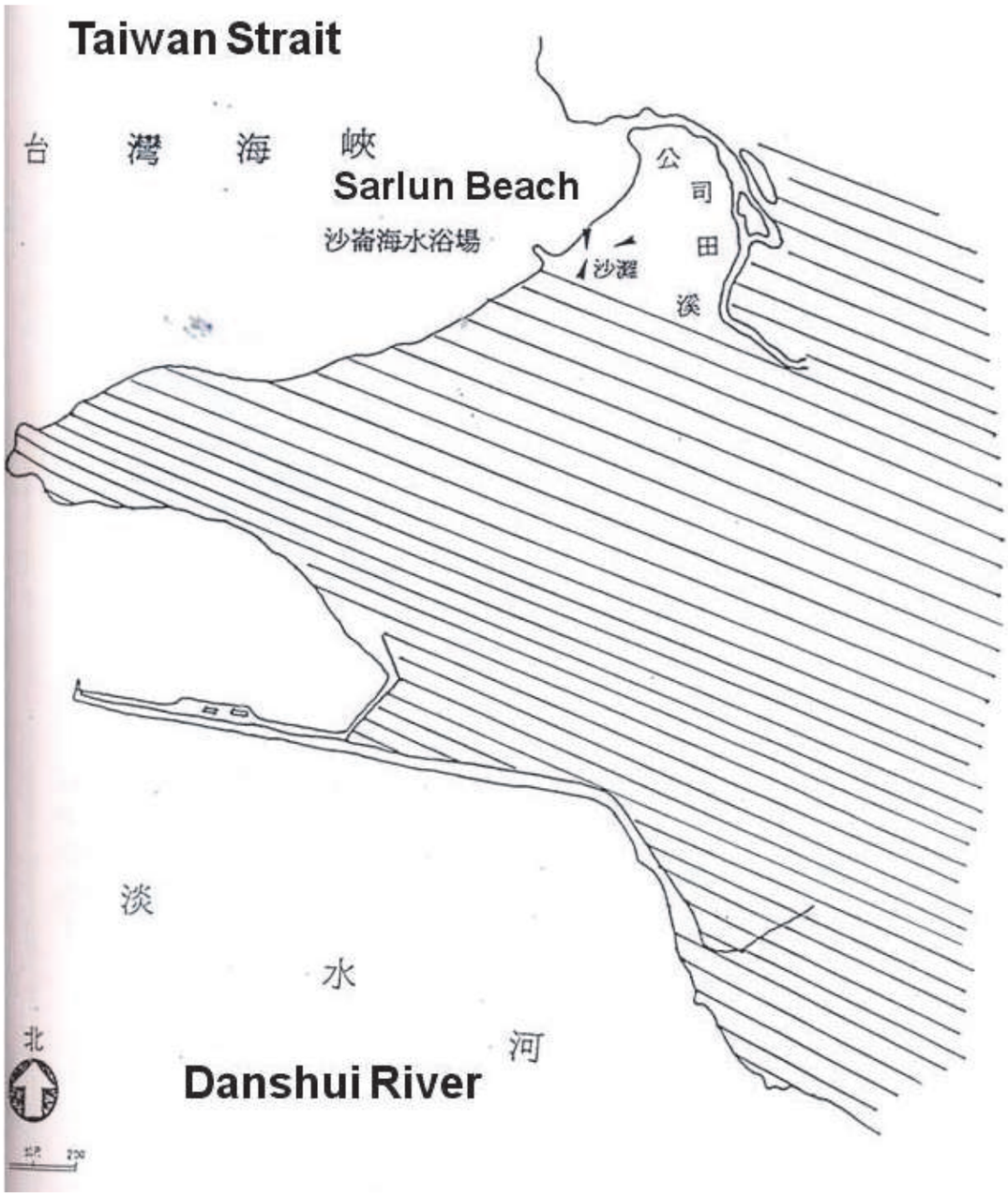

Fig. 3. Sampling sites of *Imperata cylindrica* in Sarlun, Taipei, Taiwan. Sampling sites were labeled as arrows indicated.

spermidine, 2% beta-mercaptoethanol, 2% CTAB, and 2% PVP. The homogenate was mixed well and subjected to water bath at 65℃ for 10 minutes. The mixture was then centrifuged at 13000 rpm for 15 minutes at room temperature. The upper layer was recovered to a new centrifuge tube. Equal volume of chloroform:isoamyl alcohol (24:1) was added. After thorough mixing, centrifugation was carried out at 13000 rpm for 15 minutes at room temperature. This step was repeated twice in order to remove proteins and metabolites. The upper water layer was transferred to a new centrifuge tube. One third volumes of 10 M LiCl was added, mix well and subjected to incubation under 4℃ overnight. The solution was centrifuged at 13000 rpm for 30 minutes at 4℃. The pellet was washed by 1mL 70% (v/v) ethanol. The solution was transferred to a sterilized microcentrifuge tube for centrifugation at 13000 rpm for 15 minutes at 4℃. The wash step was repeated twice to remove salt. The pellet was air dried. One hundred μL sterilized ddH_2O was added to resuspend RNA. RNA concentration was measured by NanoDrop spectrometer. The RNA integrity was checked by 2% agarose gel electrophoresis, and the solution was used as templates for reverse-transcription immediately followed by storage under -80℃.

2.5 Differential display assay

Differential display and the subsequent silver stain were carried out by following the manufacturer instruction (RNAimage Kit 5, GenHunter Corporation, Cat. No. G505; SILVER SEQUENCE™ Staining Reagents, Promega, Cat. No. Q4132). After reverse-transcription, cDNAs were used as templates to perform another PCR with AP primers provided by the manufacturer. PCR products were loaded into denature acryl-amide gel for electrophoresis at 1700V for 4 hours. The gel was stained by silver stain immediately after the electrophoresis was completed. The Air-dried gel was scanned by scanner (EPSON PERFECTION V750 PRO, EPSON). The electrophoretic bands were compared to identify gel bands specific to either Chuwei or Sarlun population.

2.6 Isolation of electrophoretic bands for DNA cloning and sequencing

The electrophoretic bands were excised from the acrylamide gel by sterilized dissection knife, and collected in 1.5 mL eppendorfs. The recovery step was conducted by use of QIAGEN II Gel Extraction Kit (QIAGEN, Cat. No. 28704). The DNA concentrations were detected by NanoDrop spectrometer. The recovered DNA was used for TA cloning. Cloning was conducted using TA Cloning Vector (RBC, # 200-09205) as cloning vector. T4 DNA ligase was used for ligation between TA vector and DNA. *E. coli* (DH5α) was used as hosts. The ligated plasmids were transformed into DH5α competent cells by heat-shock, and incubated under 37℃ on LB medium (with ampicillin) for 14 hours. Five to eight colonies were selected from each plate to perform colony PCR and subsequent sequencing.

2.7 Database searching

Sequences acquired were searched against NCBI database using BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.*, 1990). Plant Genome Database (PlantGDB, http://www.plantgdb.org/), the Gene Ontology（http://www.geneontology.org/）, the Arabidopsis Information Resource（TAIR, http://www.arabidopsis.org/）, and MIPS *Oryza sativa* database（MOsDB, http://mips.helmholtz-muenchen.de/plant/rice/）were also used. In nucleotide sequence searching, the cloned sequences were compared against databases by BLASTn program and

BLASTtx program for matches. In protein sequence searching, the acquired sequences were first translated by ExPASy Translate Tool (http://au.expasy.org/tools/dna.html), and then screen for possible protein sequences manually. The translated protein sequences were compared against databases by BLASTp program.

3. Results and discussion

3.1 Population size of Chuwei ecotype is much smaller than before

Imperata cylindrica (L.) Beauv. var. *major* (Nees) Hubb., a top-ten weed (Holm *et al*., 1977), was found in Taiwan all around the island (Hsu, 1975). In America, *Imperata cylindrica* was found as a non-endemic species dominating Florida and neighboring states in both dry and wetlands (King & Grace, 2000). Interestingly, it was found growing in Chuwei mangrove salt marsh wet land as well. To track the dynamics of population size of Chuwei ecotype, we carried out field survey since 2008. Surprising, we observed decrease of population size of Chuwei ecotype. Two of the sampling sites (Chuwei and Sarlun) we selected for the field work (site 2 and site 3 as shown in Figure 2) before year 2000 were found missing since the beginning of 2009. Only sampling site 1 (around 30 m^2) still existed so far (Figure 2). In addition, the size of sampling site 1 is only less than half of the size as before. Therefore, we concluded that Chuwei ecotype is now decreasing its population and is endangered. However, the reason why the population size decreased is unknown.

3.2 Proline content in leaves of Chuwei ecotype decreased dramatically after flooding in the field

We have two hypotheses for the interpretation of possible reasons for the population size decrease of Chuwei ecotype. The first hypothesis is supported by the evidence that more and more *Kandelia* trees are invading the habitat of Chuwei Imperata. These *Kandelia* trees may threaten the survival of Imperata. It is expected that in ten years, this area will be dominated by *Kandelia* trees and Imperata Chuwei ecotype will eventually be extinct. In order to test the second hypothesis, we compare proline content in leaves of *I. cylindricain* in the field before and after local flooding on the spring tide (high tide) date. Samples were collected at monthly interval from August to December in 1995. For proline content analysis, leaves sampling was performed with 20 individuals within a day. To test if proline content changed in response to flooding, same leaf samples were collected before and after flooding (1 hr difference) on spring-tide days. Proline content analysis showed that proline content dropped dramatically after flooding. On July 14 in 1995, proline content decreased 53 % after flooding. On August 13, proline content decreased 46 % after flooding. On October 12, proline content decreased 67 % after flooding. Moreover, on February 20 in 2011, proline content decreased 40% after flooding (Table 1).

The decrease of proline content in response to local flooding in Chuwei supported our second hypothesis. In the salt marsh wetland, high level of proline content is helpful in maintaining the osmotic equilibrium in the cells. When local flooding occurred, proline dropped rapidly, which is harmful to plants since osmotic equilibrium was not maintained in the cells. This might eventually lead to cell death. In order to determine if proline content in leaves of plantlet increases in responses to flooding, flooding treatment was performed three months in the greenhouse to see long-term effect on proline. Leaf samples were collected and analyzed. Results showed that the accumulation of proline was not found in leaves of flooding-treated plantlet in the greenhouse (data not shown).

Sampling date	Before flooding	After flooding
07.14.1995	138.99± 7.53	64.62± 1.58[1]
08.13.1995	130.94 ± 8.29	70.49± 4.84[1]
10.12.1995	156.82± 14.84	52.16± 2.57[1]
02.20.2011	150.45 ± 2.37	89.97± 3.68[1]

[1] Values showed significant difference in Student's t-test at 5% level. S.E. was at 5% level. Proline count unit: micro g gfw^{-1}

Table 1. Average proline content in leaves of *I. cylindrica* from Chuwei population before and after flooding on spring-tide (high tide) days. Sampling was performed in 1995 and 2011.

3.3 Proline serves more than one function in *Imperata cylindrica* Chuwei ecotype? - A cross-talk between flooding and salt stress

In a previous study, *I. cylindrica* from Chuwei was tolerant to 1% salt treatment. Therefore, it was grouped as a salt-tolerant ecotype (Chang & Chou, 2006). The tolerance ability was even stronger than *Kandelia candel* (tolerant to 0.8 % salt), a dominant mangrove forest species in Chuwei salt marsh wetland (Hwang & Chen, 1995). Since Chuwei sampling site is a mangrove salt marsh land, how plants survived in such a high salt land is poorly known. According to our study, proline content of *I. cylindrica* from Chuwei was much higher than those from others in the field (Chang & Chou, 2006). They were also higher under salt treatment. In the greenhouse experiments, proline of *I. cylindrica* from Chuwei was highly accumulated in response to salt treatment.

However, does proline serve only single role in plants? It has been reported that the regulation of ion uptake and production of organic solutes (*i.e.* methyl proline) is related to combined salt and flood stress tolerance in some wetland plants (Carter *et al.*, 2006). In addition, mechanism of how some halophytes tolerate flooding is reviewed (Colmer and Flowers, 2008). In present study, proline content dropped dramatically (almost 50 %) after local flooding (only in one hour) in Chuwei ecotype, suggesting that proline may serve as a signal molecule in response to flooding. The dynamic of proline in leaves of Chuwei *Imperata* was suggested which might not only be involved in salt stress, but also in flooding stress response.

The decrease of proline content in response to flooding can be explained by two mechanisms. The first mechanism is that proline acts as a direct response to flooding. It has been reported the accumulation of proline was regulated by abscisic acid (ABA) (Stewart, 1980; Stewart & Voeberg, 1985). Alcohol dehydrogenase *ADH* gene expression was also known regulated by ABA (de Bruxelles *et al*., 1996) through G-box, a promoter region (Walker *et al*., 1987; Lu *et al*., 1996). It is possible that *ADH* gene expression and proline accumulation cross-talks, and is regulated through ABA. Since proline accumulated in response to salt stress in Chuwei ecotype, decrease in proline content may be an easier way for cell to recognize to activate fermentation pathway, including activation of *ADH* gene. An alternative mechanism is an indirect response of proline to flooding. The accumulated proline transported downward toward root (shoot to root) to protect root from salt damage, and anti-transpirated with a flooding signal molecule from root to shoot (Else *et al.*, 1996) for stomata closes in response to flooding. This pathway might overlap with water-deficit pathway. Besides, a long distance transport of proline as a signal molecule was required. However, we did not confirm any of the possibility because it was very complicated to simulate a physiology experiment with two stresses acting at the same time. Timing makes the experiment even difficult to resolve. Maybe

an easier way to keep tract on proline dynamic was to label proline using isotope as a tracer. Although the role of proline as signal molecule in response to salt stress has been proposed (Maggio *et al*., 2002; Hare & Cress, 1997), the involvement of proline in flooding response has not been discovered before. Anyway, due to the fact that proline functioned in response to both flood and salt stress in present study, a crosstalk between two distinct pathways, salt and flooding response, was suggested.

3.4 Differential display reverse transcription assay revealed five genes which were differentially expressed in Chuwei ecotype as compared to Sarlun population

Differential display was first introduced In 1992, by Liang and Pardee as an easy application for comparison, identification and isolation of genes expressed as mRNA in various cells under designated conditions (Liang and Pardee, 1995). The basic steps of differential display include isolation of intact RNA from sample, reverse transcription of the RNA using an arbitrary primer to produce ssDNA, several rounds of polymerase chain reactions using the same primer to amplify the corresponding cDNAs, and display of the cDNAs as bands on a matrix gel, such as a DNA sequencing gel. In order to identify differentially expressed genes in Chuwei ecotype, differential display (DD) was introduced. The differences of gene expression between Chuwei ecotype and Sarlun population were compared. By comparing and collecting the bands uniquely appeared to either Chuwei or Sarlun populations. Population-specific ESTs was isolated and identified.

Three biological replicates were included. RNAs extracted from Chuwei ecotype and Sarlun population were subjection to QC. To check RNA integrity, RNAs extracted were used to perform electrophoresis in 2% agarose gel at 120V for 15 minutes. Differential display was conducted by three replicates with one of the replicate. Bands with high reproducibility were indicated and excised from the gel for subsequent cloning procedure. A total of twenty-six bands specific to Chuwei ecotype were detected, whereas seventeen bands were specific to Sarlun population.

Detail information about Chuwei-specific bands is listed in Table 2 and described as follow. BLAST search result of band C3 nucleotide sequence showed that part of the sequence of *Zea mays* chloroplast genome which coding for 16s rRNA was identified. This matched sequence was also found to be present in *Arabidopsis thaliana* chloroplast, *Zea perennis* mitochondrion and uncultured environmental bacterium. (Takeuchi *et al*., 2005), whereas others had no significant matches. For BLAST search result of band C12 nucleotide sequence, a candidate uncharacterized mRNA sequence of *Triticum aestivum* was identified (Kawaura *et al*., 2009). This cDNA had similar sequence matches in other plant species, including *Stellaria longipes*, *Sorghum bicolor*, *Oryza sativa*, and *Arabidopsis thaliana*. No hits were found in animals, which suggested it's a plant-specific gene. It appeared to be a putative disease resistance gene (Yang *et al*., 2008, Sequencing Project International Rice, 2005, Tanaka *et al*., 2008, Ohyanagi *et al*., 2006), but remained to be a hypothetical one (Paterson *et al*., 2009). For BLAST search result of band C12 nucleotide sequence, most ID matched hypothetical disease-resistance related genes in plants, which supported C12 to be an EST sequence of a plant-specific gene for a hypothetical protein. For BLAST search result of band C7 nucleotide and protein sequence, nevertheless no significant matches were found in NCBI, PlantGDB, TAIR and MOsDB databases.

Detail information about Sarlun-specific bands is listed in Table 3 and described as follow. BLAST search result of band S4 nucleotide sequence showed that an ATPase III subunit in *Dendrocalamus latiflorus* chloroplast, *Sorghum bicolor* chloroplast and *Zea mays* chloroplast

(Strittmauer and Kossel, 1984, Alexandrov *et al*., 2009, Wu *et al*., 2009) was identified. Moreover, BLAST search result of band S4 protein sequence showed that ATP synthase CF0 subunit III of many eudicotyledons such as *Geranium carolinianum* and *Erodium texanum* (Guisinger *et al*., 2008) was identified. For BLAST search result of band S8 nucleotide sequence, the most likely candidate was a hypothetical protein of *Sorghum bicolor* (Paterson *et al*., 2009). Similar sequences were also found in *Zea mays* and *Oryza sativa*, still hypothetical proteins (Yu *et al*., 2005, Lai *et al*., 2004). Moreover, for BLAST search result of band S8 protein sequence, eight possible queries out of six reading frames were blast against NCBI database. Five of these queries had no significant similarities, the others matched to hypothetical proteins of *Sorghum bicolor, Zea mays* and *Oryza sativa*.
For band S9 nucleotide BLAST search, a putative casein kinase of *Oryza sativa* was found. This sequence was also similar to another putative serine/threonine protein kinase of *Oryza sativa* (Sasaki *et al*., 2002). For band S9 protein BLAST search, eight possible queries out of six reading frames were submitted to NCBI database. Two of them had matches indicating serine/threonine protein kinase of *Oryza sativa*; four were found to share similarities with hypothetical proteins among various vascular plants; the rest had no significant similarities found. For band S6 nucleotide BLAST search, no significant similarities were found among all databases mentioned above. For band S6 protein BLAST search, four possible queries were submitted. No significant similarities were found.

Band #	Size (bp)	Accession #	Identity	Annotation of matched gene
C3	776	AY928077	99%	*Zea mays* chloroplast, 16S rRNA
C12	553	AK333595	82%	*Triticum aestivum*, hypothetical

Table 2. Differential display bands specific to Chuwei ecotype.

Band #	Size (bp)	Accession #	Identity	Annotation of matched gene
S4	404	AY928077	98%	*Zea mays*, ATP synthase C chain
S8	468	AK333595	96%	*Sorghum bicolor*, hypothetical
S9	383	AAT44311	79%	*Oryza sativa*, casein kinase

Table 3. Differential display bands specific to Sarlun population.

3.5 Adaptational divergence of *Imperata cylindrica* Chuwei ecotype

Imperata Chuwei ecotype encountered both flooding and salt pressure. In order to survive, plants must possess adaptation mechanism against flooding and salt stress. The increase of

ADH activity and decrease of proline content may be a mechanism against flooding stress, and accumulation of proline may be a mechanism against salt stress (Chang & Chou, 2006). The working model for flooding stress tolerance of Chuwei ecotype was proposed. Before flooding, proline content accumulated in leaves of Chuwei ecotype in response to salt stress. After flooding, leaf ADH activity increased. By contrast, leaf proline content decreased after flooding. These changes can be reached in only one hour. In addition, we identified several differentially expressed genes in Chuwei ecotype and Sarlun population in present study. All these biochemical, physiological changes and genes may contribute to the tolerance of Chuwei ecotype to both flooding and salt stresses, and therefore drove this population diverged form other populations as a distinct ecotype through evolution. However, what was the signal transduction relationship among ADH gene activation and proline, and what was the crosstalk link between flooding and salt stress response remained unknown. More studies on crosstalk of flooding and salt stress physiology of Chuwei ecotype on the signal transduction level are needed.

3.6 Sea-level rise as a potential threat to the survival of *Imperata cylindrica* ?

In present study, we observed a decrease of population size of *Imperata* Chuwei ecotype. In the proline content assay, we also observed dramatic drop of proline content after local flooding in the field in Chuwei ecotype. The drop of proline may be detrimental to plants. These results suggested that sea-level rise can be a potential threat to the survival of *Imperata* Chuwei ecotype. However, it is still not sure that the decrease of population size of *Imperata* Chuwei ecotype is directly or indirectly related to sea-level rise. What can be expected is that in the near future the population may be totally wiped out due to sea-level rise. Since Chuwei ecotype may possess many unknown and valuable salt-tolerant genes, we urge to preserve Chuwei ecotype for conservation.

3.7 Conclusions

The results from present study showed that proline content dropped dramatically after the local flooding at Chuwei. The results supported our second hypothesis that local flooding-mediated proline drop may threaten the survival of *Imperata*. However, the first hypothesis was also supported by the fact of overpopulation of *Kandelia* trees in Chuwei. A concurrent contribution of the two is suggested. In addition, differential display identified Chuwei ecotype or Sarlun population-specific transcripts in the greenhouse. These differentially expressed genes can be important in salt or flooding stress tolerance. However, further validation and confirmation of gene expression using other molecular tools is needed.

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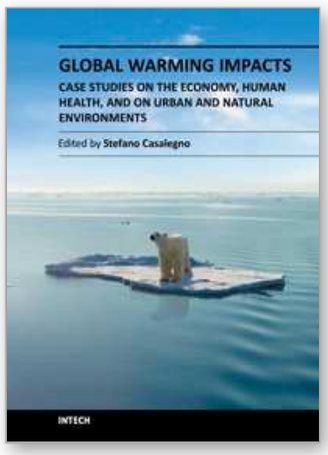

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This book addresses the theme of the impacts of global warming on different specific fields, ranging from the regional and global economy, to agriculture, human health, urban areas, land vegetation, marine areas and mangroves. Despite the volume of scientific work that has been undertaken in relation to each of each of these issues, the study of the impacts of global warming upon them is a relatively recent and unexplored topic. The chapters of this book offer a broad overview of potential applications of global warming science. As this science continues to evolve, confirm and reject study hypotheses, it is hoped that this book will stimulate further developments in relation to the impacts of changes in the global climate.

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