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Taxonomic Review of the Genus *Echinochloa* in Korea (I): Inferred from Sequences of cpDNA and nrDNA

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Abstract The genus *Echinochloa* (L.) P. Beauv. comprised of approximately 30-40 species in the tropical and warm temperate regions of the world, including numerous interspecific and intraspecific types which make the genus difficult to identify. As an attempt to identify the species within the genus easier, the taxonomy of the genus *Echinochloa*, Poaceae in Korea was reviewed on the basis of sequencing data derived from nuclear ribosomal DNA internal transcribed spacer (ITS) and external transcribe spacer and chloroplast DNA *trn*L intron, *trn*L-F intergenic spacer and *mat*K regions using a total of 46 accessions representing all the species in Korea. The results of maximum parsimony found separate lineage comprised of *E. colona* and *E. frumentaceae* which are not Korean species, but no resolution within Korean *Echinochloa* species, supporting the suggestion of Yamaguchi group that *E. crus-galli*, *E. oryzoides*, and *E. esculenta* should be considered to belong to the same species. However, the relationship between these three species and the other species, i.e. *E. oryzicola* should be better understood with more detail studies.

Key words: DNA sequences, *Echinochloa*, Phylogeny, Taxonomy

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Introduction

The genus *Echinochloa* (L.) P. Beauv. includes 30-40 species in the tropical and warm temperate regions of the world. It is a difficult genus of numerous interspecific and intraspecific types. There is no reliable morphological character to distinguish them, resulting in poor taxonomic understanding of the genus (Clayton and Renvoize, 1999). Although the two species of *Echinochoa*, *E. frumentacea* Link and *E. esculenta* (A.Braun) H.Scholz, are grown as minor cereal crops in India, China and Japan, several species are aggressive colonizers of disturbed habitats and major noxious weeds in cultivated areas of the world (De Wet et al., 1983). Especially, *E. crus-galli* (L.) P. Beauv, *E. colona* (L.) Link and *E. oryzicola* (Vasinger) Vasinger are problematic weeds in many paddy fields of Eastern Asian countries. The two species, *E. crus-galli* and *E. oryzicola* are noxious weeds in Korean agricultural fields as well. *Echinochloa crus-galli* exhibits very diverse morphological and ecological forms and includes several varieties such as var. *echinata* (Willd.) A. Chev., var. *praticola* Ohwi, var. *muticum* (Sickenb.) Simps., and var. *sieberiana* (Asch. & Schweinf.) Chevalier. The two varieties, *echinata* and *praticola*, however, have been treated as either the synonyms of var. *crus-galli* or independent varieties depending on taxonomists (The Plant List, 2014). The wide morphological and ecological diversity may be related to the allohexaploidy of the species (Yabuno, 1983).

The scientific name of *E. oryzicola* has been controversial as *E. phyllopogon* (Stapf) Stapf ex Kossenko, *E. crus-galli* var. *oryzicola* (Vasinger) T. Koyama, tetraploid *E. crus-galli* var. *oryzicola* and *E. oryzoides* (Ard.) Fritsh (Yamaguchi et al., 2005). Tabacchi et al. (2006) and Michael (2007) have, however, treated *E. oryzoides* as a different species from *E. oryzicola* by drooping panicles at maturity, lower glumes with usually 1/4-2/5 as long as the spikelets, awned lower lemmas, and the number of chromosomes, $2n = 54$, confirmed by Lee et al. (2013).

It was known that seven morphological taxa of the genus, *E. crus-galli* var. *crus-galli*, var. *echinata*, var. *praticola*, *E. oryzicola*, *E. colona* (L.) Link, *E. glabrescens*, and *E. esculenta*, are distributed throughout Korea and one more species, *E. oryzoides*, has been recently added (Lee et al., 2013). Although they provided the key to the genus *Echinochloa* of Korea,

morphological identification is very difficult because interspecific and intraspecific variations are very often observed in the open fields.

The objective of this study was to identify the taxa of *Echinochloa* and review the treatment of *E. crus-galli* varieties *echinata* and *praticola* to var. *crus-galli*. We also aim to resolve the phylogenetic relationships among the Korean *Echinochloa* taxa using molecular methods.

Materials and Methods

Sampling

Forty-six accessions of the *Echinochloa* species and one accession of *Setaria viridis* were selected for sequencing. Most of the Korean *Echinochloa* species were collected by authors from August to September of 2011 throughout the South Korea and the foreign accessions were provided by National Plant Germplasm System, USDA-ARS, National Taiwan University Herbarium (TAI) and Prof. Qiang Sheng of Nanjing Agricultural University, China. Plant materials are presented in Table 1.

DNA extraction and quantification

Genomic DNA was isolated from the green leaves of plant materials using a Genomic DNA Isolation Kit (NucleoGen, Germany) according to the instructions provided. The isolated DNA concentration and relative purity were checked using a Nanodrop ND-1000 (Dupont Agricultural Genomic Laboratory) and adjusted to 25 ng/μL for PCR amplification.

Table 1. The 46 *Echinochloa* accessions used for sequencing analyses.

ID	Taxa	Origin	ITS	ETS	trnL-F	matK
E6	*E. oryzicola*		KF010176**	KF101250	KF163558	KF010213
E7*	*E. oryzoides*	India	KF010177	KF101251	KF163559	KF010214
E8	*E. oryzoides*	Masan, Gyeongsangnamdo	KC164281	KF101252	KC164291	KC164269
E9*	*E. crus-pavonis*	Argentina	KC164274	KF101253	KF163560	KC164262
E11	*E. crus-galli*	Yeongjongdo, Inchon	KF010178	KF101254	KF163561	KF010215
E13*	*E. frumentacea*	USA	KC164278	KF101255	KC164288	KC164266
E25	*E. oryzicola*	Hongcheon, Gangwondo	KC164279	KF101256	KC164289	KC164267
E26	*E. oryzicola*	Hongcheon, Gangwondo	KF010179	KF101257	KF163562	KF010216
E27	*E. oryzicola*	Hongcheon, Gangwondo	KF010180	KF101258	KF163563	KF010217
E28	*E. oryzicola*	Hongcheon, Gangwondo	KF010181	KF101259	KF163564	KF010218
E31	*E. crus-galli*	Naju, Jeollanamdo	KF010182	KF101260	KF163565	KF010219
E38	*E. crusgalli* var. *echinata*	Naju, Jeollanamdo	KF010183	KF101261	KF163566	KF010220
E40	*E.crus-galli*	Naju, Jeollanamdo	KF010184	KF101262	KF163567	KF010221
E46	*E. oryzicola*	Gyehwado, Jeollabukdo	KC164280	KF101263	KC164290	KC164268
E53	*E. crusgalli* var. *echinata*	Seosan, Choonchungnamdo	KF010185	KF101264	KF163568	KF010222
E54	*E.crus-galli*	Seosan, Choonchungnamdo	KF010186	KF101265	KF163569	KF010223
E55	*E. oryzicola*	Choonchon, Gangwondo	KF010187	KF101266	KF163570	KF010224
E56	*E. crus-galli*	Yeongjongdo, Inchon	KF010188	KF101267	KF163571	KF010225
E58	*E. oryzicola*	Gyehwado, Jeollabukdo	KC164272	KF101268	KC164284	KC164260
E80	*E. esculenta*	Jejusi, Jejudo	KC164277	KF101269	KC164287	KC164265
E81	*E. crus-galli*	Seogwiposi, Jejudo	KC164276	KF101270	KC164286	KC164264
E88	*E. crus-galli* var. *praticola*	Naju, Jeollanamdo	KF010189	KF101271	KF163572	KF010226
E89	*E. crus-galli* var. *praticola*	Naju, Jeollanamdo	KF010190	KF101272	KF163573	KF010227
E90*	*E. crus-galli*	Phillipines	KF010191	KF101273	KF163574	KF010228
E91*	*E. colona*	China	KC164275	KF101274	KF163575	KC164263
E93*	*E.crus-galli*	China	KF010192	KF101275	KF163576	KF010229
E95*	*E.crus-galli*	China	KF010193	KF101276	KF163577	KF010230
E96*	*E.crus-galli*	China	KF010194	KF101277	KF163578	KF010231
E99*	*E. crus-galli* var. *austro-japonesis*	China	KF010195	KF101278	KF163579	KF010232

Table 1. The 46 *Echinochloa* accessions used for sequencing analyses (continued).

E100	*E. oryzicola**	China	KF010196	KF101279	KF163580	KF010233
E105	*E.crus-galli*	Cheongsong, Gyeonsangbukdo	KF010197	KF101280	KF163581	KF010234
E109	*E.crus-galli*	Gyeongju, Gyeonsangbukdo	KF010198	KF101281	KF163582	KF010235
E114	*E.crus-galli var. echinata*	Busan	KF010199	KF101282	KF163583	KF010236
E115	*E.crus-galli var. echinata*	Busan	KF010200	KF101283	KF163584	KF010237
E116	*E.crus-galli*	Busan	KF010201	KF101284	KF163585	KF010238
E117	*E.crus-galli*	Busan	KF010202	KF101285	KF163586	KF010239
E120	*E.crus-galli*	Busan	KF010203	KF101286	KF163587	KF010240
E121	*E.crus-galli*	Busan	KF010204	KF101287	KF163588	KF010241
E123	*E.crus-galli*	Jecheon, Choongcheonbukdo	KF010205	KF101288	KF163589	KF010242
E125	*E. oryzicola*	Gwangyang, Jeollanamdo	KF010206	KF101289	KF163590	KF010243
E130	*E.crus-galli var. praticola*	Namhae, Gyeongsangnamdo	KF010207	KF101290	KF163591	KF010244
E132	*E.crus-galli var. echinata*	Jangseong, Jeollanamdo	KF010208	KF101291	KF163592	KF010245
E133	*E.crus-galli*	Sooncheon, Jeollanamdo	KF010209	KF101292	KF163593	KF010246
E137	*E.crus-galli var. praticola*	Sancheong, Gyeongsangnamdo	KF010210	KF101293	KF163594	KF010247
E138	*E. oryzicola*	Paju, Gyeonggido	KF010211	KF101294	KF163595	KF010248
E140	*E. oryzicola*	Wonju, Gangwondo	KF010212	KF101295	KF163596	KF010249

*Foreign origin
**Genbank accession number

Sequencing and Phylogenetic analysis

The cpDNA, *mat*K and *trn*L intron and *trn*L-F intergenic spacer, and nrDNA ETS and ITS regions were PCR amplified using primers provided in Table 2. Details of PCR amplification reactions and purification were the same as described in Lee and Hymowitz (2001). DNA sequencing was conducted at the Genotech, Daejeon, Korea. The analyzed DNA sequences were aligned with Clustal W with manual editing and gaps were positioned to minimize nucleotide mismatches if necessary. Some ambiguously aligned sequences were not included in the phylogenetic analysis. Pairwise nucleotide differences of unambiguously aligned positions were determined using the distance matrix option in PAUP* 4.0b 10 (Swofford, 1998). All gaps were treated as missing data. Data sequences have been submitted to the National Center for Biotechnology information (NCBI) and obtained GenBank numbers.

Maximum parsimony analyses of ITS, ETS, *trn*L-F and *mat*K were performed individually and in combination using PAUP* 4.0b 10. A heuristic search was carried out with 100 random addition replicates and tree bisection-reconnection (TBR) branch swapping. The options with MULPARS, STEEPEST DESCENT, COLLAPSE, and ACCTRAN optimization were selected to search for the most parsimonious trees. Bootstrap analysis (Felsenstein, 1985) of 1000 replicates was performed using MEGA 5.2 (Tamura et al., 2011) with simple addition and TBR branch swapping options to evaluate the degree of support for each branch. The amount of

Table 2. Primers and reaction conditions used in this study.

Seqeuncing regeions	Primers	Primer sequences	Annealing temperatures (°C)	References
ITS	ITS4 ITS-Y5	TCCTCCGCTTATTGATATGC TAGAGGAAGGAGAAGTCGTAACAA	57	White et al. (1990) Kita & Ito (2000)
ETS	RETS4_F 18S_R	TGGCTACGCGAGCGCATGAG AGACAAGCATATGACTACTGGCAGG	55	Gillespie et al. (2010) Starr et al. (2003)
trnL-F	Trn-F Trn-C	ATTTGAACTGGTGACACGAG CGAAATCGGTAGACGCTACG	54	Taberlet et al. (1991) 〃
*mat*K	Trn3914F P1 W trnK-2R	 TACCCTATCCTATCCAT AACTAGTCGGATGGAGTAG	55-50 touch down	Johnson / Soltis (1994) Designed in this study Hilu et al. (1999) Kelly Steele (1991)

Table 3. Sequence characteristics of the ITS, ETS, ITS+ETS, *trn*Lintron and *trn*L-F intergenic spacer region, *mat*K and *trn*L-F+*mat*K in 46 taxa of the *Echinochloa*. Outgroup, *Setaria viridis*, was not included for sequence characteristics.

Characteristics	ITS	ETS	ITS + ETS	*trn*L-F	*mat*K	*trn*L-F + *mat*K	All combined
Length range (bp)	585-589	448-449	1034-1038	823-829	2373-2376	3196-3205	4230-4241
Aligned length (bp)	590	450	1040	857	2389	3244	4283
Sequence divergence (%)	0-4.06	0-3.45	0-3.69	0-0.49	0-.0.68	0-0.6	0-1.31
No. of excluded sites (%)	0	0	0	12(0.37)	0	12	75(1.75)
No. of included sites (%)	590	450	1040	845(98.6)	2387	3232(99.6)	4208(98.3)
No. of indels	5	1	6	9	5	14	20
No. of variable sites	105(17.8)	94(20.89)	199(19.13)	44(5.2)	83(3.48)	127(3.93)	364(8.65)
No. of informative sites (%)	31(5.25)	76(16.89)	150(14.42)	40(4.73)	20(0.84)	24(0.74)	75(1.78)
No. of autapomorphic sites (%)	74(12.54)	18(4.0)	49(4.71)	4(0.47)	63(2.64)	103(3.19)	289(6.87)
CI of parsimony informative site	0.9310	0.9510	0.9276	1.0000	0.9655	0.9773	0.9331

phylogenetic information in the parsimony analysis was estimated using the consistency index (Kluge and Farris, 1969) and retention index (Farris, 1989). Distance trees based on combined sequencing data were constructed using the neighbor-joining method (Saitou and Nei, 1987) using PAUP* 4.0b 10 (Swofford, 1998). The numbers of nucleotide substitutions (excluding gaps) were estimated using the two-parameter method of Kimura (1980).

Results

Forty-seven samples, examining for *mat*K, *trn*L intron and *trn*L-F intergenic spacer, ETS, and ITS sequence represent seven species of Korean and foreign origins and an allied species as the outgroup. Characteristics of aligned sequences are provided in Table 3.

cpDNA sequence analyses

The sequence lengths were 823-829 bp in *trn*L-F, 2373-2376 bp in *mat*K and 3196-3205 bp in *trn*L-F + *mat*K. They were aligned into 857 bp, 2389 bp and 3244 bp, respectively, of which 44 bp, 83 bp, and 127 bp were variable and 4 bp (0.47%), 63 bp (2.64%), and 103 bp (3.19%) were parsimony informative. For the ingroup, direct pairwise distance ranged from identity to 0.49%, 0.68%, and 0.6% in *trn*L-F, *mat*K and *trn*L-F + *mat*K between *E. colona* and several samples, respectively. The heuristic search identified 16,383 equally parsimonious trees of 132 steps with consistency indices of 0.8966 (excluding uninformative characters) and 0.9773 (including uninformative characters) and a retention index of 0.9845 using *trn*L-F + *mat*K. Bootstrap values ranged from 54 to 100%.

nrDNA sequence analyses

The lengths of the ITS, ETS, and ITS + ETS were 585-589 bp, 448-449 bp, and 1034-1038 bp, then aligned into 590 bp, 450 bp, and 1040 bp, respectively. The number of variable sites was 105 bp, 94 bp, and 199 bp, of which 31 bp (5.25%), 76 bp (16.89%), and 150 bp (14.42%) were parsimony informative sites. Direct pairwise distance ranged from identity to 4.06%, 3.45%, and 3.69% between *E. colona* and *E. crus-galli*, respectively. Parsimony analysis of 47 ITS + ETS using equally weighted character states resulted in 30,300 parsimonious trees. The trees have a length of 221 steps, consistency indices of 0.7647 (excluding uninformative characters) and 0.9276 (including uninformative characters) and a retention index of 0.8865. Bootstrap values ranged from 63 to 100%.

Combined cpDNA and nrDNA analysis

The length of all combined data were 4,230-4,231 bp, then aligned into 4,283 bp, of which 364 bp were variable and 75 bp were parsimony informative. Maximum parsimony analysis produced 7,693,100 trees of 478 steps. Consistency indices of 0.7117 (excluding uninformative characters) and 0.9128 (including uninformative characters) and a retention index of 0.8865. Bootstrap values ranged from 65 to 100%. Direct pairwise distance ranged from identity to 1.31% between the clade of *E. colona* and *E. frumentacea* and *E. crus-pavonis.*

Phylogenetic resolution

The strict consensus trees obtained from cpDNA and nrDNA are shown in Fig. 1. The genus *Echinochloa* was monophyletic in both trees. The *Echinochloa* was consisted of two main lineages in both trees, although the clustering was different. One clade (I) was consisted of *E. crus-pavonis*, *E. crus-galli*, *E. escuenta* and *E. oryzoides* and the other clade (II) was consisted of the rest of the examining taxa, *E. frumentacea*, *E. colona, E. oryzicola,* and *E. crus-galli*, in the cpDNA tree. On the other hand, *E. frumentacea* and its progenitor *E. colona* were located at the basal to all other taxa in the nuclear DNA tree. *Echinochloa crus-pavonis*, clustered

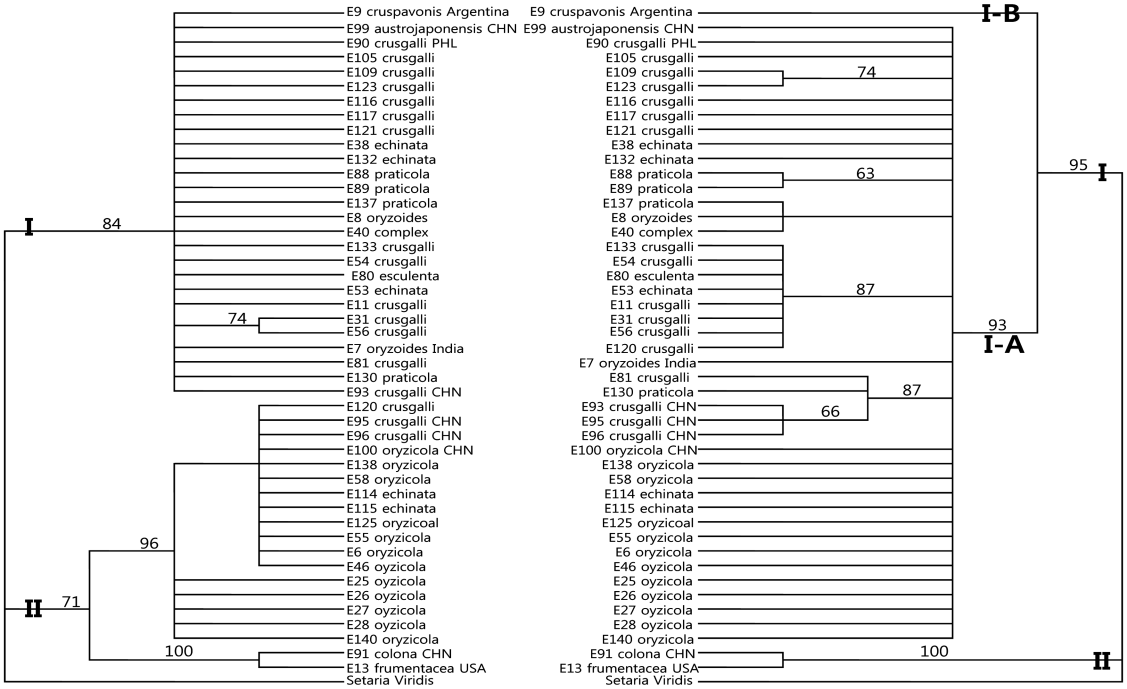

Fig. 1. Maximum parsimony tress derived from chloroplast DNA *trn*L intron, *trn*L-F intergenic spacer and *mat*K and nuclear ribosomal DNA internal transcribed spacer (ITS) and external transcribe spacer regions using a total of 46 accessions. Left is a strict consensus of 16,383 maximum parsimonious trees based on cpDNA (tree length = 132, Consistency index (CI) = 0.9773, and Retention index (RI) = 0.9845). Right is a strict consensus of 30,300 maximum parsimonious trees based on nrDNA (Tree length = 221, Consistency index (CI) = 0.9276, and Retention index (RI) = 0.8865). The numbers above the branches indicate a bootstrap value of >50%.

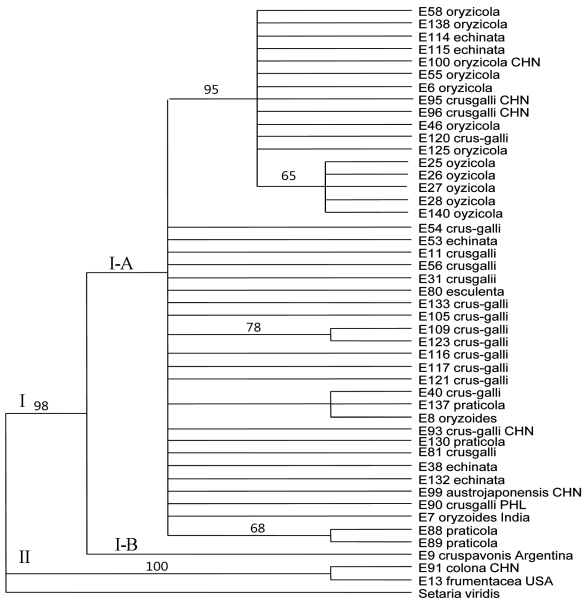

Fig. 2. A strict consensus of 7,693,100 maximum parsimonious trees derived from all combined data set. Tree length is 478 steps. Consistency indices of 0.7117 (excluding uninformative characters) and 0.9128 (including uninformative characters) and a retention index of 0.8865. The numbers above the branches indicate a bootstrap value of >50%. Bootstrap values ranged from 65 to 100%.

with *E. crus-galli*, *E. escuenta* and *E. oryzoides* in the strict consensus tree based on cpDNA solely consisted of a subclade in clade I in the tree based on nrDNA.

The strict consensus tree of all combined markers showed the similar topology to that derived from nrDNA (Fig. 2). It was consisted of two lineages, one was a clade of *E. frumentacea* and *E. colona* (II) and the other lineage was consisted of the others (I). The large clade (I) comprising the *E. crus-pavonis, E. oryzicola, E. crus-galli, E. oryzoides,* and *E. esculenta* was supported by a high bootstrap, 98%. *Echinochloa crus-pavonis* solely consisted a subclade (I-B) within the clade I. The subclade I-A was comprised of *E. oryzicola, E. crus-galli, E. oryzoides,* and *E. esculenta* which are treated as crus-galli complex by Aoki and Yamaguchi (2008). *Echinochloa oryzicola* with a few accessions of *E. crus-galli*, however, consist a independent subclade with a high bootstrap value of 95%, within the clade I-A. Intersetingly, several accessions of *E. oryzicola* (i.e; E25, 26, 27, 28, and 140) formed a lineage in the combined analysis while they were not monophyletic in both cpDNA and ITS sequences-derived analyses.

All the distance trees (not shown) inferred from the neighbor-joining analyses of each cpDNA, nrDNA, and all combined regions were congruent with the maximally parsimonious trees. There was no clustering difference at all between the neighbor-joining and parsimonious trees.

Discussion

Although genus *Echinochloa* has received attention among agronomists due to its properties of the noxious weeds in many agricultural fields, overall comprehensive taxonomic study is still lacking. As an ongoing effort to determine the extent of morphological and phylogenetic relationships among *Echinochloa* species in Korea, we produced phylogeny of *Echinochloa* in Korea based on cpDNA and nrDNA sequences. Due to low levels of DNA sequence variations among species in both loci, the phylogenetic relationships were not fully resolved among closely related species.

The results of phylogenetic analyses using cpDNA and nrDNA sequences showed slightly different relationships with positions of *E. crus-pavonis* and a lineage of *E. colona* and *E. frumentacea*. *Echinochloa crus-pavonis* coming from Argentina was clustered with *E. crus-galli*, *E. escuenta*, and *E. oryzoides* in one of main clades of cpDNA strict consensus tree whereas solely consisted a subclade within clade I in nrDNA strict consensus tree. This is not congruent with the result of the study conducted by Yamaguchi et al. (2005) which included Nigerian diploid. Nigerian *Echinochloa crus-pavonis* was consisted of the sole clade in the strict consensus tree constructed using non-coding region sequences of *trn*T-L-F of cpDNA. Argentinian *E. crus-pavonis*, however, was nested within the crus-galli complex in cpDNA trees (Aoki and Yamaguchi, 2008), which was congruent with the previous results. Aoki and Yamaguchi (2008) supposed that *Echinochloa crus-pavonis* described as a South American representative species (Michael, 2007) was clustered with the other South American *E. crus-galli*, implying a genetic difference between the American and Eurasian *E. crus-galli*. However, it may be reasonable to infer that this clustering was more related to phylogeographic relationships than to a genetic difference suggesting their different evolutionary process. More detailed phylogeographic stuty of *E. crus-pavonis* and *E. crus-galli* including extensive sampling from the American countries will help reveal the relationships.

Independent lineage of *E. colona* and *E. frumentacea* was strongly supported with 100% bootstrap in both trees. *Echinochloa frumentacea* is annually cultivated mostly in India and Central Africa. It is known to have been domesticated recently from *E. colona* (Clayton and Renvoize, 1999). *Echinochloa colona* is an annual weed widely distributed throughout the subtropical and tropical regions of Asia, Africa, and America. It is a hexaploid with 2n = 6x = 54, but differs from *E. crus-galli* in genomic constitution (Yabuno, 1966, 1983). The close phylogenetic relationship between *E. colona* and *E. frumentacea* was also supported by previous studies (Yamaguchi et al., 2005; Aoki and Yamaguchi, 2008). Among *E. oryzicola, E. crus-galli complex, E. crus-pavonis*, and a clade of *E. colona-frumentacea*, the phylogenetic relationship was not resolved enough using cpDNA non-coding region sequences of *trn*T-L-F in the study of Yamaguchi et al. (2005). However, a clade of *E. colona - frumentacea* was a basal group in both the cpDNA and nrDNA trees of Aoki and Yamaguchi's study (2008) and in the nrDNA tree of this study. Interestingly, a clade of *E. colona - frumentacea* showed a close relationship with *E. oryzicola* clade with high bootstrap, 71% in the cpDNA tree of our study. This result may indicate that *E. frumentacea* and *E. colona* share the same maternal genetic background with *E. oryzicola* (2n = 4x = 36), but not with crus-galli complex, when we consider the hypothesis that *E. frumentacea* and *E. colona* does not share the same genomes with *E. esculenta* and *E. crus-galli* (Yabuno, 1966).

It has been known that three botanical varieties of *Echinochloa crus-galli*, var. *crus-galli*, *echinata* and *praticola* are distributed in Korea. However, taxonomic treatment of these botanical varieties are not recognized as the distinct taxa by taxonomists who has a broad view of the species due to continuous morphological variations. *Echinochloa crus-galli* is allohexaploid (6X) and known to be composed of paternal donor of *E. oryzicola* and unknown maternal diploid (Aoki and Yamaguchi, 2008). *Echinochloa oryzoides*, distributed in Korea, China, Japan and North America and also hexaploid with 2n = 54, is known to share the same genome with *E. crus-galli* (Yabuno, 1984). *Echinochloa esculenta*, cultivated mostly in temperate regions in Korea, China, and Japan, is also hexaploid with a chromosome number of 2n = 6x = 54. This species is known to have been domesticated from *E. crus-galli* (Yabuno 1966). Phylogenetic relationships among Korean *Echinochloa*; i.e. *E. crus-galli* including var. *crus-galli*, *echinata* and *praticola*, *E. oryzicola*, *E. esculenta*, and *E. oryzoides*, were remained poorly resolved in both analyses due to very low sequence variation with only 32 nucleotide substitutions out of 4,284 sequences. The average pairwise-distance values among Korean *Echinochloa* were 0.2% for ETS, *mat*K, and *trn*L-F and 0.4% for ITS. One difference between the cpDNA and nrDNA trees is an independent clustering of *E. oryzicola* with high bootstrap, 96%, in the cpDNA tree, but no relationship resolved in the nrDNA tree. Especially, relationship between *E. oryzoides*, *E. esculenta*, and *E. crus-galli* which are morphologically diverse, could not be discerned in this study due to limited resolutions in cpDNA and nrDNA sequences. These three species also showed the same sequences in the *trn*T-L-F coding regions using East Asian accessions in a molecular phylogenetic study of wild and cultivated *Echinochloa* conducted by Yamaguchi et al. (2005). Yamaguchi group supposed that these three species, called as a crus-galli complex, be considered to belong to the same species with more diverse studies including Isozyme analysis (Nakayama et al., 1999) and polymerase chain reaction-restiction fragment length polymorphism (PCR-RFLP) technique (Yasuda et al., 2002). The results of our study support their suggestion,

especially in Korean *Echinochloa*.

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