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Molecular phylogeny of the Chinese weedy rice based on nrDNA ITS and two cpDNA sequences

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Abstract

Direct sequencing data from the nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) region and two chloroplast DNA (cpDNA) fragments (*psbA-trnH* and *petG-trnP*) of Chinese weedy rice (*Oryza stavia* L. f. sp. *spontanea*) were analyzed separately and simultaneously in the MEGA 6.0 software. The nrDNA, cpDNA and cpDNA+nrDNA phylogenetic trees showed that the weedy rice collected from Northern China, *Oryza sativa Japonica* group and *Oryza rufipogon*, were on the one phylogenic node whereas weedy rice were collected from Southern China and *Oryza sativa Indica* group were on the other phylogenic node. The results revealed that there were geographical and *indica-japonica* differentiations in Chinese weedy rice which were corresponding to their geographical distribution and cultivated rice (*Oryza sativa*) *indica-japonica* differentiation. These results demonstrated that Chinese weedy rice from different regions have diverse origins and they were the relative origin group of the cultivated rice as there is no wild form available in Northern China. Additionally, clone sequence variation of the nrDNA ITS regions in intra-individual weedy rice provides evidence origin of other *Oryza* taxa through the process of hybridization or introgression. Data from this research further confirmed that the Chinese weedy rice was evolved from local rice varieties (as the female parent) which naturally hybridized with other *oryza* materials.

Key words: Weedy rice, nrDNA ITS region, *psbA-trnH* and *petG-trnP* intergenic spacer, molecular phylogeny, hybridization

Introduction

Weedy rice is widely distributed and is a troublesome weed of paddy rice worldwide, particularly where direct seeding or no-till technology is applied for rice farming (Burgos et al. 2014; Juan et al. 2014). In comparison to other weeds, it is very difficult to get away from this weed because of its similar morphological features with cultivated rice. To date, no herbicides are available to prevent or control the weedy rice (Bhagirath et al. 2014; Li et al. 2014). As a notorious weed occurring in rice fields, weedy rice causes significant yield reduction of rice and affects the quality of rice grains because of its high competitive ability and persistence in rice fields (Zhang et al. 2012). Nonetheless, the weedy rice is considered to be a useful reservoir of genetic variation for rice improvement because it is successfully adapted to the natural growing conditions and has many useful genes (Subudhi et al. 2014).

The weedy rice is traditionally classified as the same species as Asian cultivated rice (*Oryza sativa* L.) based on its morphological attribute and it also possess the same ‘AA’ genome type (2n=24). Therefore, weedy rice was nominated as *Oryza sativa* f. sp. *spontanea* (Juan et al. 2014; Li et al. 2014). However, the origin of weedy rice is still unclear and remains controversial. At present, three hypotheses have been proposed regarding the origin of weedy rice: 1) adaptation of wild rice varieties to cultivation in order to survive from the human disturbance, including some individuals with wild characters; 2) the creation of hybrids by *Oryza sativa* L. spp. *indica* Kato and *O. sativa* L. spp. *japonica* Kato. or the formation of hybrids between cultivated rice and wild rice; 3) de-domestication of cultivated rice to a wild form. Each hypothesis can be supported by certain lines of evidence (Zhang et al. 2012; Subudhi et al. 2014).

A clear understanding of the origin and evolution of weedy rice will be helpful to design an effective management to control this weed (Qiu et al. 2015).

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Recently, due to the fast development of technologies for DNA sequencing, robust DNA molecular tools have been applied to analyze both chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) sequences, and they are also widely used in plant molecular systematic studies to infer phylogenetic relationships (Appelhans et al. 2014). Although the internal transcribed spacers (ITS) of nrDNA are relatively short with about 600 bp in size, they have been employed to resolve phylogenetic problems among genera, species and populations for their rapidly evolving rate (Sebastian et al. 2014). Additionally, the nrDNA ITS has been very useful to identify progenitors of hybrid taxa due to its bi-parental inheritance nature (Ahmad et al, 2014; Zhang et al. 2014). The intergenic spacer *psbA-trnH* and *petG-trnP* are in the most variable non-coding regions in the chloroplast genome, and the most extensive uses of these spacers were in lower-level plant systematics, particularly at the species level (Yang et al 2014). The cpDNA is predominantly transmitted through the seeds in most angiosperms including the *oryza* and usually exhibits geographically structured variation. It is widely used for phylogeny analysis at a lower taxonomic level (Shannon and Jeff 2014; Wang et al. 2014). However, it is known that phylogenetic hypotheses based on a single gene or character may not represent true organism relationships and single DNA sequence probably restricted reality and reliability in the study. The combined use of nuclear and chloroplast DNA (cpDNA) data would help researchers to comprehensively understand the origin and evolutionary history of the weedy rice (Shin et al 2014; Carlos et al. 2015).

There are a few reports available on the fast and reliable nucleotide sequence for the weedy rice evolution analysis so far. In this study, the nrDNA ITS and two cpDNA (*psbA-trnH* and *petG-trnP*) sequences were analyzed and used to reveal the origin and evolutionary history of Chinese weedy rice, which would be beneficial to better understand, control and utilize genetic resources of Chinese weedy rice.

Materials and methods

Plant materials

Weedy rice is found in many rice planting areas in China. Thus, in order to validate and further explore the origin of different weedy rices, geographically diverse accessions were obtained from primary rice growing area in the Southern and Northern China from 2009 to 2011. A total of 60 weedy rice accessions distributed in rice fields were collected during an extensive field survey from different provinces *viz*., Liaoning, Jilin and Heilongjiang in Northern China and Hubei and Jiangsu province in Southern China. Most of the weedy rice grain from Southern China were long and thin with *Indica* rice characteristics. In contrast, the primary weedy rice grain from Northern China was short and round with *Japonica* rice characteristics. Samples of cultivated rice varieties and common wild rice accessions from different origins were assembled and included in this study. Additionally, the *Echinochloa crusgalli* was selected as outgroups because of its close relationship with rice. All seeds of rice accessions were planted in fields in Shenyang Agricultural University (SYAU). The information of the rice accessions are listed in Table 1.

DNA amplification

To reveal the Chinese weedy rice origin, we selected and sequenced the ITS of nrDNA and *psbA-trnH* and *petG-trnP* of chloroplast region for phylogenetic analyses. PCR amplification of the nrDNA ITS region, including ITS1, the 5.8S rDNA gene, and ITS2, was performed using primers ITS-1 (5'-GGAAGTAAAA GTCGTAACAAGG-3') and ITS-4 (5'-TCCTCCGC TTATTGATATGCTTAA-3') which was based on rice rDNA sequences. The *psbA-trnH* intergenic spacer region was PCR amplified using the primers psbAf (5'-GTTATGCATGAACGTAATGC TC-3') and trnHr (5'-CGCGCATGGTGGATTCACAATCC-3'). The *petG-trnP* intergenic spacer region was PCR amplified using the primers petGr (5'-GGTCTAATTCCTATAACTTT GGC-3') and trnPf (5'-GGGATGTGGCGC AGCTTGG-3').

DNA sequencing

To ensure the sequences accuracy of the nrDNA ITS and cpDNA regions, bidirectional sequencing were conducted by the Beijing genomics institute (BGI, Beijing, China). Direct sequencing primers were the same as those used for the PCR. All sequences generated in this study were deposited in the National Center for Biotechnology Information (NCBI).

Sequence alignment and phylogenetic analysis

Bidirectional sequences were assembled using the program SeqMan Pro (DNASTAR, Lasergene) and then aligned by MEGA 6.0 software. The boundary of the nucleotide sequences ITS regions (including ITS1, 5.8S rDNA, and ITS2) and those of the *psbA-trnH* and *petG-trnP* regions were determined in comparison with

Table 1. Information of the rice accessions or varieties used in this experiment

Accession no.	Collection region or name of variety	Distribution and Taxon	
WR1-1-5	Shenyang City, Liaoning Province (LNSY)	Northern China	Weedy rice (*Oryza sativa* f. sp. *spontanea*)
WR2-1-5	Tieling City, Liaoning Province (LNTL)		
WR3-1-5	Anshan City, Liaoning Province (LNAS)		
WR4-1-5	Dandong City, Liaoning Province (LNDD)		
WR5-1-5	Panjin City, Liaoning Province (LNPJ)		
WR6-1-5	Liaoyang City, Liaoning Province (LNLY)		
WR7-1-5	Yinkou City , Liaoning Province (LNYK)		
WR8-1-5	Fuxin City, Liaoning Province (LNFX)		
WR9-1-5	Yanbian City, Jilin Province (JNYB)		
WR10-1-5	Jiamusi City, Heilongjiang Province (HLJJMS)		
WR11-1-5	Huanggang City, Hubei Province (HBHG)	Southern China	Weedy rice (*Oryza sativa* f. sp. *spontanea*)
WR12-1-5	Jiangsu Province (JS)		
OR1	Boluo County Guangdong Province	Common wild rice (*Oryza rufipogon*)	
OR2	Huilai County Guangdong		
OR3	Dongxiang County, Jiangxi Province		
OR4	Gaozhou City Guangdong Province		
OI1	93-11 (the typical *indica* rice variety)	Cultivated rice (*Oryza sativa* L. ssp. *indica*)	
OI2	Minhui63		
OJ1	Shennong265 (SN265)	Cultivated rice (*Oryza sativa* L. ssp. *japonica*)	
OJ2	Nipponbare (the typical *japonica* variety)		
OG	*Echinochloa crusgalli*	Outgroup	

several published sequences in GenBank.

Congruence between the nuclear and chloroplast loci was evaluated using the incongruence length difference (ILD) test as implemented in PAUP4.0. Phylogenetic trees were constructed according the separately and simultaneously analysis of ITS and cpDNA regions with the neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) approaches. They are implemented with the computer program MEGA 6.0.

ITS clone sequencing

The direct sequencing methods can conveniently obtain the exactly nrDNA ITS sequences, however, there exist overlap peaks in sequence diagram. Thus the clone sequencing was done in partial weedy rice to verify the bases additively for hybridization. The purified DNA fragments of the ITS regions were then cloned to the pMD18-T Simple Vector (TakaRa, Dalian, China). Eight selected positive clones for each accession were chosen and sent to BGI for inserted ITS region sequencing using SP6/T7 primers, then the different ITS repeat types could be identified.

Results

Phylogenetic analysis of nrDNA ITS data

A continuous sequence covering ITS1, 5.8S and ITS2 was successfully obtained for all newly sequenced rice materials. The ITS sequences for the weedy rice have not been recorded before. The boundaries of the weedy rice ITS regions were determined and spliced by comparison with the sequence of *Oryza rufipogon* (AY749361.1). The results showed that the length of ITS obtained in current study ranged from 587 to 589 bp among the rice materials with an average length of 588 bp. ITS1 length varied from 193 to 194 bp while ITS2 length ranged from 230 to 232 bp in weedy rice. In all weedy rice examined, the 5.8S rDNA sequence had a constant length of 164 bp with highly conservation, and no mutations were found within this segment. It was, therefore, assumed that all of these ITS sequences were functional orthologues rather than paralogues. Within the whole region, ITS segments were highly variable, 34 variable positions (17.5%) and 25 informative sites (12.9%) were found in the ITS1 sequences, while 45 variable positions (19.6%) and 32 informative sites (13.9%) were recorded in the ITS2 sequences. The G+C content of ITS1 were about 72.16% to 72.68% and that of ITS2 ranged from 75.86% to 76.52%, while the content G+C of 5.8S was 59.15% (97 bp) but lower than the non-coding regions. The sequence similarity between pairwise sequences was 99.3% to 100% in the weedy rice, the results showed that the weedy rice ribosomal genes were highly conserved and the intraspecific genetic structure were relatively stable.

The clustal W results showed that the nrDNA ITS sequences of the weedy rice obtained from the same collection region were absolutely identical, suggesting that there was no intraspecific sequence variation and hence, only one sequence was selected from the same collection region for further phylogeny analysis. A total of 39 sequences including these sequences and the ITS sequence downloaded from NCBI nucleotide database were used to construct nrDNA ITS matrix. The multiple aligned nrDNA ITS matrix, consisted of 647 bp, of which 224 (34.6%) sites were variable and 101 (15.6%) sites were parsimony informative. The phylogenetic analysis results showed that the tree topologies generated by NJ using MEGA 6.0 software were almost fully congruent with those generated by MP and ML. They separated all the weedy rice into two distinct groups (Fig. 1). Interestingly, all of the weedy rice accessions from Northern China were grouped with *Oryza rufipogon* and *Oryza sativa* L. *japonica* subspecies (SN265 and Nipponbare) which geographically located in the North-eastern part of its range, while the weedy rice from Southern China was separately grouped with the *Oryza sativa* L. *indica* subspecie commercial rice varieties (93-11 and Minhui 63) which are geographically located in the south-eastern part of its range. This result indicates that there were obviously *indica-japonica* differentiations in weedy rice which correspond to their geographical distribution. The weedy rice accessions were more closely related to cultivars from Northern China, an area with no acknowledged record of wild rice cultivation. The phylogenetic analyses suggested that weedy rice from different collection places should have diverse origins and weedy rice in China probably originated directly from local rice cultivars through rice reversion mutation, de-domestication or hybridization.

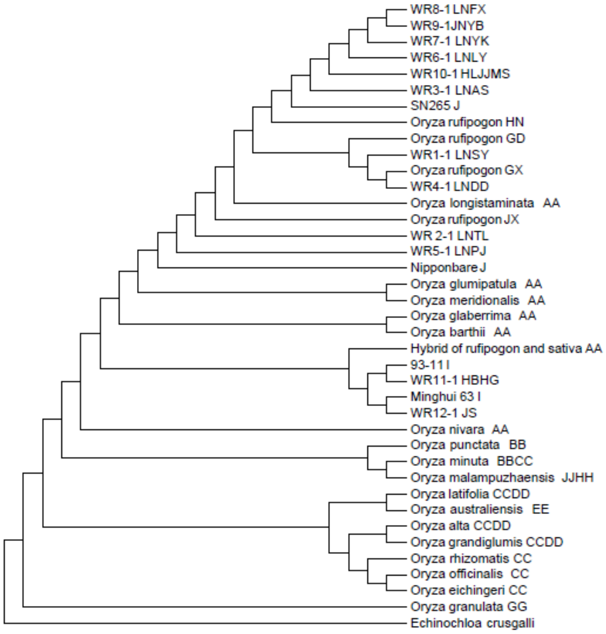

Fig. 1. The phylogenetic tree of Chinese weedy rice inferred from nrDNA ITS data using the neighbor-joining method with 1000 bootstrap replicates

Phylogenetic analysis of the combined cpDNA data

PCR products of the *psbA-trnH* and *petG-trnP* regions were successfully amplified and directly sequenced. The boundaries of the *psbA-trnH* and *petG-trnP* regions were determined and spliced in comparison with the sequence of *Oryza rufipogon* (GU575260.1 and KF562709.1, respectively). The length of *psbA-trnH* and *petG-trnP* ranged from 531 to 532 bp and 242 to 243 bp among the *oryza* material individuals, respectively.

The two cpDNA regions were combined into a single data set for further phylogenetic analyses because the chloroplast genome is not subjected to recombination. Further, the result of the ILD test (P = 0.9) indicated that the two cpDNA regions were not significantly different from one another. The contribution of each region to the length of combined matrix was *psbA-trnH* of 532 bp and *petG-trnP* of 243 bp, respectively. A low number of polymorphic sites were detected in cpDNA. The *psbA-trnH* and *petG-trnP* regions were not downloaded correspondingly for all included taxa. The aligned combined cpDNA matrix only including the sequences obtained here, consisted of 778 bp, of which 68 (8.7%) were variable and 29 (3.7%) were potentially phylogenetically informative.

cpDNA phylogenetic trees inferred using the different methods were congruent in general topology, and were almost consistent in topology by nrDNA ITS (Fig. 2). This result demonstrated that there was obvious cpDNA *indica-japonica* differentiations in weedy rice and suggested that the weedy rice from the different regions has different origins based on the chloroplast DNA analysis and its origin strongly associated with the local rice cultivars.

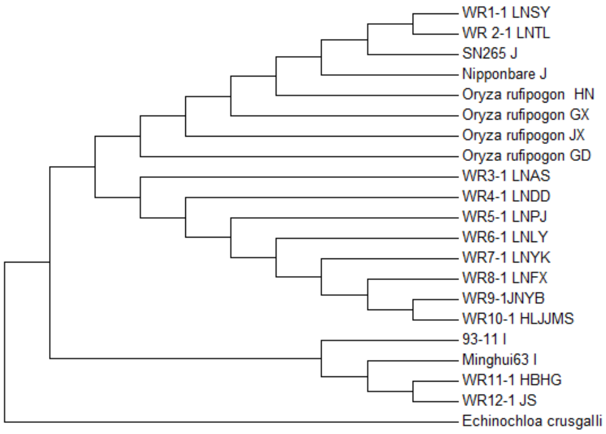

Fig. 2. The phylogenetic tree of Chinese weedy rice inferred from combined cpDNA *psbA-trnH* and *petG-trnP* data using the neighbor-joining method with 1000 bootstrap replicates

Combined analysis of nrDNA and cpDNA data

The ILD test indicated that the chloroplast and the nuclear data were fully congruent (P = 0.67), and it is therefore, justifiable to combine them in a single data set for further analysis and unveiling the origin of weedy rice. Therefore, we combined the two data sets in favour of a more robust phylogenetic hypothesis and the combined data matrix consists of 21 accessions. Sequence variation in the ITS regions was much higher than that was observed in cpDNA. Compared to ITS data set, the cpDNA data contained fewer potentially parsimony-informative characters and provided less phylogenetic resolution. The combined dataset comprised 1428 bp, of which 294 (20.6 %) sites were variable and 131 (9.2 %) were parsimony informative. The combined data set yielded a similar topology as observed with nrDNA or plastid DNA. The combined analyses supported that there was a close relationship between the Chinese weedy rice and the cultivated rice. In conclusion, the combined tree was better resolved than any of the single plastid or nuclear trees.

Clone sequencing

The PCR amplification fragments of ITS were easily sequenced, however, direct sequencing revealed additional signals in their respective chromatograms, hinting the presence of divergent ITS paralogues. The additive peaks (two different alleles in a diploid genome) during the direct sequencing of PCR products were well differentiated between cloned sequences. Thus, eight clones for each weedy rice accession were sequenced and 2 individual clones from the same PCR product were sequenced to avoid mutation introduced by Taq polymerase. The results revealed that all the polymorphic sites by direct sequencing were confirmed by additivity of base pairs among the eight clone sequences (Fig. 3).

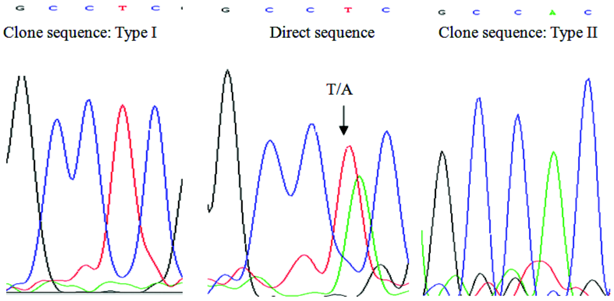

Fig. 3. The partial chromatogram comparison results of direct and clone sequencing

On the basis of ITS clone sequence, the intra-individuals can be divided into two types: type I 179 bp in ITS1 regions for A; Type II 179 bp in ITS1 regions for T. All 8 individuals of the cultivated rice and the wild rice had identical ITS sequences for type I or type II. The results showed that the intra-individual ITS sequences of the weedy rice showed heterogeneity and provided further evidence for the hybrid or introgressed origin. Because plastid DNA is maternally inherited and nrDNA ITS is bi-parentally inherited, it was suggested that cultivated rice *japonica* (in Northern China region) or the *indica* (in Southern China region) was the maternal parent in all original hybridization with other *oryza* materials.

Discussion

Knowledge of the phylogenetic and evolutionary relationship of weedy rice is of great importance for the purpose of control and utilization of the existing populations (Li et al. 2014). Many previous studies of weedy rice populations have indicated its very close relationships with cultivated rice. Those findings demonstrated that weedy rice tends to converge morphologically with rice varieties grown in the same field after a few generations. Such rapid evolution of ''crop mimicry'' of weedy rice is most likely via introgression of alleles from the cultivated rice, facilitating the adaptive evolution of weedy rice under natural and human selection (Song et al. 2014; Thurber et al. 2014). Recent studies, particularly ones based on DNA analysis, revealed that the features of *indica* and *japonica* have been found in the wild relatives of *Oryza sativa* (Qiu et al. 2015). Such genetic differentiation of weedy rice populations is likely to be associated with the introgression from their two locally corresponding subspecies of rice, *indica* and *japonica* (Craig et al. 2014; Shin et al. 2014).

Because of the favourable climate, only *Oryza sativa japonica* is suitably growing in Northern China, while farmers in Southern China primarily cultivate *Oryza sativa indica*. Southern China is an important distribution area of *Oryza rufipogon* while Northern China is an area with no history record of wild rice cultivation, so it is widely accepted that weedy rice from Northern China does not have any direct relationship with wild rice (Zhang et al. 2012). Weedy rice in Southern China is more likely to be similar to *indica*, while weedy rice in Northern China is more likely to be similar to *japonica* (Sun et al. 2013; Qiu et al. 2015). In the present study, the results indicated that weedy rice differentiation and origin with obvious geographical phenomenon was associated with the selected regions in China.

In case of worldwide weedy rice, hybridization was suggested as the most likely event in its origin (Li et al. 2014; Subudhi et al. 2014). For example, Qiu et al. (2015) reported that the weedy rice accessions from the lower Yangtze region of China, that originated from hybridization between *indica* and *japonica* rice based on 46,005 SNPs. To our knowledge, this analysis of the origin of weedy rice by means of sequences of nrDNA ITS and chloroplast DNA is for the first time. Sequences of nrDNA ITS and cpDNA can complement each other and the combination of them can improve the ability to discriminate at the species or population level due to their different pattern of inheritance (Shin et al 2014; Carlos et al. 2015). Here, the multi-locus DNA sequences provided a similar grouping pattern for the Chinese weedy rice accessions. Phylogenetic and clone sequencing analyses strongly support a hybrid origin for Chinese weedy rice and concludes that the Chinese weedy rice is not monophyletic in its origin.

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References

Ahmad R. H., Ali O. B., Mohammad M.V., Kamil C. and Salih T. 2014. Phylogenetic relationships among *Artemisia* species based on nuclear ITS and chloroplast *psbA-trnH* DNA markers. Biologia, **69**(7): 834-839.

Appelhans M. S., Wen J. and Wagner W. L. 2014. A molecular phylogeny of *Acronychia*, *Euodia*, *Melicope* and relatives (*Rutaceae*) reveals polyphyletic genera and key innovations for species richness. Mol. Phylogenet. Evol., **79**: 54-68.

Bhagirath S. C., Anuruddhika S. K., Manoja S. W., Sakinda D. K. and Upali B. W. 2014. Effect of rice establishment methods on weedy rice (*Oryza sativa* L.) infestation and grain yield of cultivated rice (*O. sativa* L.) in Sri Lanka. Crop Prot., **55**: 42-49.

Burgos N. R., Singh V., Tseng T. M., Young N. D., Huang Z. Y. and Hyma K. 2014. The impact of Herbicide-resistant rice technology on phenotypic diversity and population structure of United States weedy rice. Plant Physiol., **166**: 208-1220.

Carlos C., Alejandro H., Luis A. and Dora F. 2015. DNA barcodes in Fig cultivars (*Ficus carica* L.) using ITS regions of ribosomal DNA, the *psbA-trnH* spacer and the *matK* coding sequence. Am. J. Plant Sci., **6**: 95-102.

Craig S. M., Reagon M., Resnick L. E. and Caicedo A. L. 2014. Distributions at hybrid incompatibility loci facilitate the potential for gene flow between cultivated and weedy rice in the US. PLoS ONE, doi:10.1371/journal.pone.0086647.

Juan E. R., Victoria B. and Fernando P. V. 2014. One-step, codominant detection of imidazolinone resistance mutations in weedy rice (*Oryza sativa* L.). Electron. J. Biotechn., **17**: 95-101.

Li X. Y., Qiang S., Song X. L., Cai K., Sun Y. N., Shi Z. H. and Dai W. M. 2014. Allele types of *Rc* gene of weedy rice from Jiangsu Province, China. Rice Sci., **21**(5): 252-261.

Qiu J., Zhu J. W., Fu F. Ye C. Y., Wang W. D., Mao L. F., Lin Z. X., Chen Li., Zhang H. Q., Guo L.B., Qiang S., Lu Y. L. and Fan L. J. 2015. Genome re-sequencing suggested a weedy rice origin from domesticated *indica-japonica* hybridization: a case study from southern China. Planta, doi 10.1007/s00425-014-2159-2.

Sebastian G., Julian R. S. and Matthias H. H. 2014. Parallel and convergent diversification in two northern hemispheric species-rich Carex lineages (*Cyperaceae*). Org. Divers. Evol., **14**(3): 247-258.

Shannon C. K. and Jeff J. D. 2014.Molecular phylogenetics of Amorpha (*Fabaceae*): An evaluation of monophyly, species relationships, and polyploid origins. Mol. Phylogenet. Evol., **76**: 49-66.

Shin H., Oh S. H., Lim Y. S., Hyun C. W., Cho S. H., Kim Y. I. and Kim Y. D. 2014. Molecular evidence for hybrid origin of aster *Chusanensis*, an endemic species of ulleungdo, Korea J. Plant Biol., **57**: 174-185.

Song B. K., Chuah T. S., Tam S. M. and Olsen K. M. 2014. Malaysian weedy rice shows its true stripes:wild *Oryza* and elite rice cultivars shape agricultural weed evolution in Southeast Asia. Mol. Ecol., **23**: 5003-5017.

Subudhi P. K., Singh P. K., Deleon T., Parco A., Karan R., Biradar H., Cohn M. A. and Sasaki T. 2014. Mapping of seed shattering loci provides insights into origin of weedy rice and rice. J. Hered., **105**(2): 276-287.

Sun J., Qian Q., Ma D. R., Xu Z. J., Liu D., Du H. B. and Chen W. F. 2013. Introgression and selection shaping the genome and adaptive loci of weedy rice in northern China. New Phytol., **197**: 290-299.

Thurber C. S., Reagon M., Olsen K. M., Jia Y. L. and Caicedo A. L. 2014.The evolution of flowering strategies in us weedy rice. Am. J. of Bot., **101**(10): 1737-1747.

Wang G. Y., Meng Y., Huang J. L. and Yang Y. P. 2014.Molecular phylogeny of *Ophiopogon* (*Asparagaceae*) inferred from nuclear and plastid DNA sequences. Syst. Bot., **39**(3): 776-784.

Yang P., Li X. W., Zhou H., Hu H., Zhang H., Sun W., Wang Y. T. and Yao H. 2014. Molecular identification of Chinese *Materia Medica* and its *Adulterants* using ITS2 and *psbA-trnH* barcodes: A case study on *Rhizoma Menispermi*. Chinese Med., **5**: 190-198.

Zhang D. Q., Duan L. Z. and Zhou N. 2014. Application of DNA barcoding in *Roscoea* (*Zingiberaceae*) and a primary discussion on taxonomic status of *Roscoea cautleoides* var. *Pubescens*. Biocheml. Syst. Ecol., **52**: 14-19.

Zhang L. J., Dai W. M., Wu C., Song X. L. and Qiang S. 2012. Genetic diversity and origin of *Japonica*- and *Indica*-like rice biotypes of weedy rice in the Guangdong and Liaoning provinces of China. Genet. Resour. Crop Evol., **59**: 399-410.