GENE FLOW AND INTROGRESSION ANALYSIS FROM RICE INTO WILD/WEEDY RELATIVES IN CENTER OF DIVERSITY IN TROPICAL AMERICA

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INTRODUCTION

Weedy rice (commonly known as red rice) is sympatric with the rice crop. In tropical America, the weedy rice complex is broadly diverse and maybe composed by various *Oryza* species (mostly annual and diploid, AA genome) that yet have not been fully identified. Weedy rice usually has feral traits (tall plants, awn seeds with red pericarp, and shattering), with varying degree of sexual compatibility and flowering overlap with the crop in different environments (Oka and Chang, 1961). Weedy rice appears to be the main candidate for gene flow and introgression from cultivated rice, since it is compatible and usually intermingled with the crop. This research is part of a project which main goal is to generate baseline genetic information for the development of guidelines on the safe introduction and use of novel agriculture traits (biotechnology derived or not native from the place of introduction), while reducing potential environmental impact on native biodiversity in the Neotropics. This work summarizes the use of molecular markers to assess and trace gene flow / introgression from transgenic and non-transgenic rice into weedy rice in confined experimental field plots and at landscape level in farmers commercial fields.

MATERIALS AND METHODS

Tracking gene flow from transgenic and non-transgenic rice into weedy rice under experimental field conditions. Weedy rice accessions representing the diversity of types found in Colombian farmer fields were used to conduct gene flow analysis, and to identify indicators for easy monitoring of genetic introgression in the crop-weedy rice complex. Gene flow was assessed using as pollen donor a transgenic line carrying the RHBV-N protein-transgene conferring resistance to RHBV, and the *gus* and *hph* (hygromycin resistance) marker genes, and a non-transgenic rice variety locally known as Purple, characterized by having purple leaves, tillers, and grain apiculus, and dominant inheritance of anthocyanins

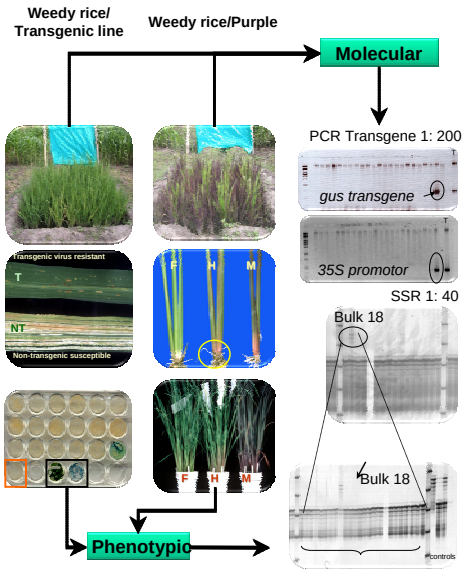

Figure 1. Analysis of gene flow at the phenotypic and/or molecular level

Gene flow was traced first at the phenotypic level by detecting *gus* expression (from transgenic plants) or anthocyanin presence (from non-transgenic plants) in plant tissues, and putative hybrids were then confirmed by microsatellite (SSR) molecular markers, or alternatively directly at the genetic level without knowing the phenotypic profile by bulking DNA samples of various plants and analyze with the specific SSRs. PCR analysis was conducted to trace the presence of transgenes and SSR analysis to confirm the hybrid nature of recovered F1 plants (Figure 1).

Tracking gene flow at landscape level in farmers fields. Herbicide resistance in rice derived from mutagenesis (imidazolinone resistance) was bred and released as improved variety Clearfield CF205® in Central America and Colombia. The herbicide resistant rice is a convenient model because of the easy tracking for the resistance trait in the weedy rice complex. Herbicide is used as a form of chemical control for the weed (positive selection). In the case of wild relatives of rice (*Oryza* genus) populations found in natural environments and in contact crop zones no herbicide is applied (neutral selection). Total of 499 accessions of weedy rice were collected from farmers fields prior and after planting 1 cycle of Clearfield CF205® tolerant to the herbicide Imazapic (IMI) in Jamundi, Valle del Cauca, and 501 accessions after planting 2-3 cycles of the variety in Tolima, (Colombia) (Fig. 2A). Flowering panicles of weedy rice in contact with flowering panicles of CF205® were randomly selected and collected (Fig. 2B). The acetolactate synthase (ALS) gene that confers resistance to the imazapic herbicide was sequenced and gene specific primers were designed to detect the single point mutation (single nucleotide polymorphism, SNP) in the ALS gene, and used to trace this gene in the weedy populations at landscape level using molecular bulk analysis (Fig. 3)

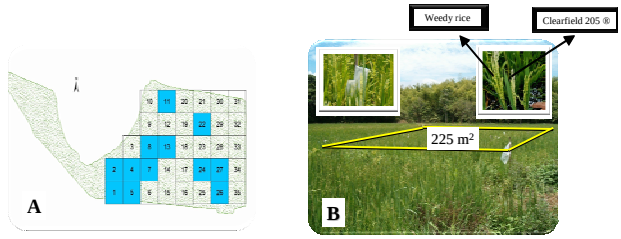

Figure 2. Sampling weedy rice from commercial plots planted with CF205®. Blue squares represent the sampled areas within a plot.

RESULTS AND DISCUSSION

Tracking gene under experimental field conditions and commercial farmers fields. Hybridization rates of about 0.01% to 0.3% confirmed by microsatellite (SSR) markers were obtained when either transgenes or the anthocyanin marker genes were used to trace gene flow under confined experimental conditions in about 24,000 derived progeny plants. All transgenic hybrids confirmed by SSR express *gus* gene and display the region of the promoter 35S CaMV.

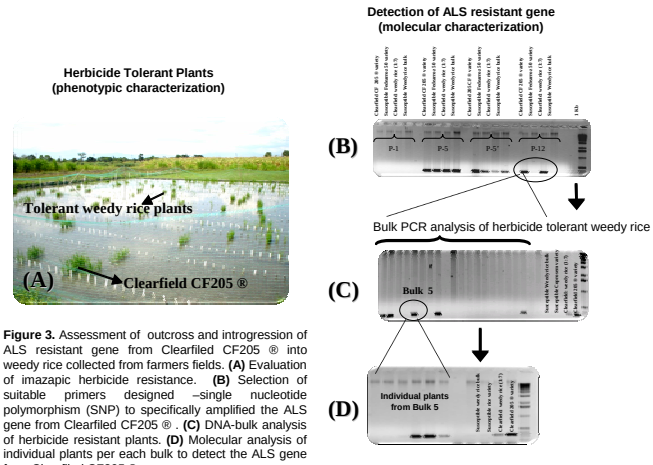

Figure 3. Assessment of outcross and introgression of ALS resistant gene from Clearfiled CF205 ® into weedy rice collected from farmers fields. **(A)** Evaluation of imazapic herbicide resistance. **(B)** Selection of suitable primers designed –single nucleotide polymorphism (SNP) to specifically amplified the ALS gene from Clearfiled CF205 ® . **(C)** DNA-bulk analysis of herbicide resistant plants. **(D)** Molecular analysis of individual plants per each bulk to detect the ALS gene from Clearfiled CF205 ®.

Progeny (49.866 plants) derived from weedy rice panicles collected in commercial Clearfield CF205® fields were first assayed for herbicide resistance in replicated field trials. DNA of herbicide resistant plants was bulked, analyzed to detect the presence of the ALS resistant gene (Fig 3 A), and subsequently to identify the number of individual plant (s) per bulk containing the ALS mutation (Fig. 3B, 3C). In the case of Valle del Cauca fields which had been planted just 1 cycle with CF205®, 9.3 % (17.566 plants) of the samples showed resistance to imazapic herbicide, but only 0.4% of these plants contained the ALS mutation from CF 205® confirming the outcross with weedy rice. The rest of the plants maybe indicative of cross-resistance to other ALS target herbicide resistance. This outcross rate is in accordance to previous results shown herein under controlled experimental conditions, and elsewhere.

CONCLUSIONS

•Outcross of ≤0.5 % is predominantly from non-transgenic or transgenic rice into weedy rice under controlled experimental conditions. Similar rates were obtained in the first cycle of outcross at landscape level in Valle del Cauca.

•The use of non-transgenic herbicide resistance as a model will give information on impact for introgression of non-transgenic resistance genes that may affect fitness of derived hybrids, invasiveness, population dynamics and genetic structure of the corresponding wild/weedy population, and for anticipating a potential impact from a transgenic situation. This information will be useful for *in situ* conservation, and could be applicable to develop guidelines for environmental safety and co-existence of different types of agriculture systems in the Neotropics.

•Using bulk DNA and PCR-based analysis allows the assessment of large number of samples with a high precision to detect hybrids. This methodology is useful for tracking and monitoring gene flow at large scale in farmers' fields and in crop-to-wild contact zones. The scoring of phenotypic trait alone (i.e. gus expression, vegetative tissue color, herbicide resistance) could either under or overestimate the level of hybridization rate.

•SSR can be used to determine gene flow but the population genetic structure needs to be known prior the analysis. In our study, thus methods and tools need to be adapted to assess out-cross at landscape level using specific SNP molecular markers detecting the gene of interest by bulk analysis, allowing to analyze large populations of samples (about 19.128 plants) in about 1 month. This protocol will also be applicable for analysis at ecological level.

REFERENCES

Oka , H. and Chang, W. 1961. Evolution 15: 418-430.