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 have not been fully elucidated. It is characterized by feral traits (tail 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/introgresion 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 farmers 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 35S (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.

RESULTS AND DISCUSSION

Tracking gene flow 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 transgenics 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 CAM.

Molecular analysis of Expressed gene from Clearfield 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% (117,566 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. 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 site 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