Development of a Novel Plant Transformation Method for Selected Crops

EUU Rathnathunga[1], PS Warakagoda[2] and S Geekiyanage[1]
[1]Dept of Agric Biology, [2]Dept of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya

Abstract

Genetic transformation of crops is a straight forward method in crop improvement. *Agrobacterium* is the unique cellular organism which is capable of transferring genetic material to plants. Callus or explant transformation followed by transgenic plant regeneration is the most common *Agrobacterium* mediated transformation method. We introduced an alternative novel*Agrobacterium* mediated transformation method through seedling transformation. *Flowering locus T* (*FT*) and *VlmybA2* for induction of early flowering and purple pigmentation respectively were used to check the effectiveness of seedling transformation method for selected monocot and dicot crops (rice, anthurium and 3 *Citrus*sp.) by*Agrobacterium*. Rice seedlings of 10cm, *Citrus* seedlings less than 1cm and anthurium seedlings of 2cm height were used for transformation. A few albino plants were observed in putative chimeric rice seedlings of 35S: *FT* and 35S:*VlmybA2*. Purple pigmentation was clearly visible only on base of the stem of putative *VlmybA2* rice seedlings in contrast to controls. Putative *VlmybA2 Citrus* seedlings produced purple pigmentation after 2 weeks of transformation. They could not survive due to contamination. During the limited experimental period of 2 months, signs of early flowering (shortened internodes and early axillary buds) were observed in *Citrus* plants. Significant growth retardation was observed in putative chimeric anthuriumseedlings transformed by 35S: *FT* and 35S:*VlmybA2* after one month of transformation. Soil isolates of above plants did not reveal the *Agrobacterium* indicating the potential of this method for bio safety. Based on the above results, seedling transformation could be effective for *Citrus* and rice. This experiment is continued with further testing for development of the seedling transformation method as an alternative novel method.

Key words: Alternative transformation method, Anthurium, Citrus, rice

Introduction

Genetic transformation of crops is a straight forward method in crop improvement.*Agrobacterium* is the unique cellular organism which is capable of transferring genetic material to plants (Mehrotra and Goyal, 2012). Although monocotyledons are not natural hosts of *Agrobacterium*, several monocots have been transformed successfully (Schlappi and Hohn ,1992 and Chan et al, 1992). Callus transformation followed by *in vitro* plant regeneration is the general method of transgenic plant production. This method takes time and sometimes not suitable for the crops that are difficult to regenerate. Pérez-Piñeiro et al, (2012) have attempted to understand the regulatory process of Agrobacterium-mediated transformation using different artificial intelligence approaches In this experiment we introduceda novel *Agrobacterium* mediated method for plant transformation through seedling transformation. We have selected two genes,*Flowering locus T* (*FT*) and *VlmybA2*, for this study. *FT* gene could induce early flowering in most of the crops. *VlmybA2* could induce purple pigmentation in several monocots and dicots.

Anmyb-related transcription factor gene ofthe anthocyanin biosynthetic pathway, *VlmybA2*, fromthe Kyoho grape (*Vitislabruscana*) was introduced into rice, *Citrus* and anthurium under the control of the cauliflower mosaic virus 35S promoter.

The *Flowering locusT* (*FT*) proteinis a mobile signal for flowering initiation.It was introduced into rice, *Citrus* and anthuriumplants under the control of the cauliflower mosaic virus 35S promoter.

Materials and Methods

Explants used were as follows:

1. *In vitro* grown anthurium seedlings of 2cm of height (around 6months in culture.

2. Traditional rice variety Deveraddili.
3. Commercially available *Citrus* spp.(Nasnaran, Heennaran andJamanaran)

Transformation method

1. Rice and *Citrus*seeds were dipped for overnight in fungicide (10mg/l of Topsine).and surface sterilized by immersing in95% ethanol for 2 minutes followed by 3 washes with distilled water. Surface sterilization was continued with 20% commercial bleach for 10 minutes and follow up washes.. Seeds were germinated on wet sterile tissue papers.
2. The seedlingswere transformed with *Agrobacterium* carrying pBE2113/*FT*and *AgrobacteriumVlmybA2*
3. After 1 week plants were transferred to pots.

Result and Discussion

Albino plants were observed in putative chimeric seedlings transformed by 35S: *FT* and 35S:*VlmybA2* after 5 days of transformation of Deveraddili. There were 6 plants out of 14 putative *FT* transformants while there were 3 albino plants out of 15 putative *VlmybA2* plants. Albino plants indicate the success of transformation as such albino plants have been observed in our previous work with rice callus transformation.

Purple pigmentation was not very clear on putative *VlmybA2* seedlings of Deveraddili. However, purple colour at the base of the plants was prominent compared to putative *FT* and control plants.

Mud from pots of above constructs and isolates of putative transformants were streaked on LB medium. Mud streaks and plant isolates were cultured on LB medium. Putative *Agrobacterium* colonies appeared on LB with plant isolates. Absence of bacteria in mud would be a useful indicator for determination of bio safety issues of rice transformed by this method in the long run.

Purple pigmentation was clear on putative *VlmybA2* seedlings of *Nasnaran* after 2 weeks of transformation, but they could not survive due to the excess growth of bacteria in seedlings.

An axillary shoot was observed in putative chimeric seedling transformed by 35S: *FT* after 3 weeks of transformation of *Nasnaran*.

During the limited experimental period of 2 months, signs of early flowering (shortened internodes and early axillary buds) were observed in *Citrus* plants.

Growth retardation was observed in putative chimeric seedlings transformed by 35S: *FT* and 35S:*VlmybA2* after one month of transformation of anthurium.

Conclusion

Seedling transformation would be a potential method for *Citrus*and rice.

References

Chan MT, Lee TM and Chang HH 1992 Transformation of indica rice (Oryza sativa L.) Mediated by Agrobacterium tumefaciens. Plant & Cell Physiology, 33, 577–583.

Mehrotra S and Goyal V 201) *Agrobacterium*-Mediated Gene Transfer in Plants and Biosafety Considerations. ApplBiochem Biotechnol.DOI 10.1007/s12010-012-9910-6

Pérez-Piñei P, Gago J, Landín M and Gallego PP 2012 Agrobacterium-mediated transformation of wheat: general overview and new approaches to model and identify the key factors involved.Transgenic plants—advances and limitations chapter 1. YeldaOzdenCiftci (ed). doi:10.5772/1409

Schlappi M and Hohn B 1992 Competence of immature maize embryos for Agrobacterium medium gene transfer. The Plant Cell, 4, 7–16.