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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :7.816(2021); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:10, ISSUE:7(10), July: 2021
Online Copy of Article Publication Available: www.ijmer.in
Digital certificate of publication: http://ijmer.in/pdf/e-Certificate%20of%20Publication-IJMER.pdf
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 4th July
Publication Date:30th July 2021

DOI: http://ijmer.in.doi./2021/10.07.205

IN VITRO PROPAGATION OF LUFFA CYLINDRICA

[1]Padala Thirupathi, [2]Dr.T. Christopher Reuben and [3]Dr. Rajender Gandu
[1]Research Scholar, [2]Assistant Professor and [3]Dr.Rajender Gandu
[1&2]Department of Botany and [3]School Assistant,
[1&2]Kakatiya University and [3]ZPHS
[1&2]Warangal and [3]Narsimhulupet, Mahabubabad
Telangana India

Abstract

We exploited the potential of cucurbits for ectopic gene expression. Agroinfiltration is a simple and commonly used method to obtain transient expression of foreign genes in plants. In contrast to in vitro transformation techniques, agroinfiltration can be used for genetic modification of mature plant tissues. Our research has shown that mature leaves of Luffa cylindrica L. (luffa), in contrast to other cucurbit species, can be successfully transiently transformed with Agrobacterium tumefaciens. We efficiently transformed luffa leaves with a reporter gene encoding β-glucuronidase (GUS). The GUS activity in transiently transformed leaf tissues was detected within 24 h after the infiltration with bacteria. The occurrence of gynoecism in sponge gourd [Luffa cylindrica (Roem.) L.] is very rare, although population with high propagation of pistillate flowers and vegetables has been developed and utilized for hybrid development. The gynoecious and male sterile populations are always been desired by the breeders across the globe. We identified a cluster bearing sponge gourd genotype VRSG-52-1 and from this genotype of sponge gourd we isolated Four sponge gourd plants viz. Gy25S, Gy27S, Gy28S and Gy29S with absolute expression of gynoecism. The researchers have been able to develop and maintain Four populations with a very high proportion of pistillate flowers from these gynoecious plants through sib-pollination. Finally, this research paper to be critically analysed about Invitro Propagation of Luffa Cylindrica

Keywords: Sponge Gourd, Gynoecism, Pistillate Luffa Cylindrica, Cucurbits, Agroinfiltration, Plants Over Expression, Monocious Plants.

Statement of the Problem

The Cucurbitaceae species (cucurbits) are a significant source of food and substances of medical importance. Several species of Cucurbitaceae, for example Cucurbita maxima, Cucurbita pepo, Cucurbita ficifolia or Cucumis sativus, are widely used as model plants for research, especially in studies of phloem functions and Botany.

The use of cucurbits in phloem research was primarily associated with the ease of exudate sampling from severed stems and petioles. The fluid exuding from the cucurbit vascular tissue contains phloem sap derived from the fascicular phloem as well as from the extrafascicular phloem. This is a spatially distinct system of sieve elements occurring specifically in Cucurbitaceae species. Cucurbit exudates are rich in proteins (their concentration can reach up to 60–100 mg/mL). It was shown, that the exudate proteins applied to C. maxima cotyledons trafficked symplastically through mesophyll plasmodesmata and translocate over long distance in the phloem. Various techniques, like the intergeneric grafting experiments or the studies on systemic movement of plant virus particles and phloem tracers, e.g., 5(6)-carboxyfluorescein or fluorescein diacetate, were used to investigate the phloem loading mechanisms and the long-distance trafficking of molecules in cucurbits.

There are only a few reports in which transgenic cucurbit species were used for investigation of phloem function. In contrast, the techniques enabling the induction of heterological expression in plant tissues are widely used as research tools for phloem studies of many non-cucurbit species, especially the plants defined as apoplastic loaders.

Agroinfiltration belongs to the most popular plant transformation techniques. In this method, the suspension of Agrobacterium, carrying a binary vector with a targeted transgene, is pressed into the intercellular spaces of a selected plant tissue, usually a leaf. Thus, the successful introduction of bacterial suspension is one of the most important requirements affecting the efficiency of transient transformation using agroinfiltration. The agroinfiltration technique was well-developed for model plants such as Nicotiana benthamiana , Nicotiana tabacum or Arabidopsis thaliana . This method was also applied in several crops, e.g., tomato (Lycopersicon esculentum Mill.), lettuce (Lactuca L.), potato (Solanum tuberosum L.) and grape (Vitis L.) Nevertheless, to date, there is no agroinfiltration protocol for generation of plant expression systems based on mature cucurbit tissues. Therefore, the potential application of various cucurbit species for a gene functions assay using agroinfiltration was tested in this research study.

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We showed that mature leaves of Luffa cylindrica L., in contrast to other cucurbits (sponge guard) tested, can be efficiently agroinfiltrated. We detected the expression of a gene encoding GUS driven by the CaMV 35S promoter in luffa leaves a few hours after agroinfiltration. Moreover, the GUS activity was also detected in the EDTA-exudates collected from the cut petioles of the transiently transformed luffa leaves.

Sponge gourd [Luffa cylindrica (Roem.) L.] is an important cucurbit vegetable grown in Asian continent. It belongs to the family Cucurbitaceae. In India it is widely cultivated and you can find it growing at almost every roof top/backyard in the rural areas. Sponge gourd is grown for its fleshy, immature, non-bitter fruits and natural sponge. Mostly eaten as cooked vegetable curries. Early and total yield have been among the major breeding objectives. In India, other than improved sponge gourd genotypes many landraces are also being grown in different parts having wide variation in shape, size, color and maturity. The landraces are grown by farmers using their own saved seeds. The Luffa genotypes are of various flowering habits, i.e., monoecious and trimonoecious, andromonoecious, gynoecious and hermaphrodite. The occurrence of gynoecism in sponge gourd is rare, although populations with high proportions of pistillate flowers have been developed and utilized for hybrid development. In this research paper, we discuss the development of sponge gourd (Luffa cylindrica (Roem.) L.) populations with a very high proportion of pistillate flowers with better agronomic performance.

MATERIAL AND METHODS

A cluster bearing sponge gourd genotype VRSG-52-1 originated from an open pollinated land race collected from Karimnagar and Nizamabad, Telangana State of India, and from this genotype of sponge gourd we isolated four sponge gourd plants viz. Gy25S, Gy27S, Gy28S and Gy29S with absolute expression of gynoecism during 2008-09. During the summer season (February sown) of 2009, four gynoecious plants were obtained in the plot of VRSG-52-1. In June 2009 planting, segregation in the F1 generation for gynoecious and monoecious plants was observed due to the existence of heterozygous gene(s) for gynoecism in the utilized male plants. It was concluded that the gynoecism trait in the identified plants was heritable and under the control of certain major recessive genes. In a F1 cross, developed using Gy25S (gynoecious plant obtained in VRSG-52-1 population) and VRSG-52-1 (monoecious plant). One monoecious plant (with 85.4% pistillate flowers) was obtained and selfed. The pollen of this monoecious plant was utilized for sib mating to one gynoecious plant (100% pistillate flowers) obtained in the same cross. During October, 2019 the selfed F2 and full sib (F2) progenies were raised under poly house conditions. Five F2 and one F2 sib plants were selected for further advancement. Observations on number of staminate and pistillate flowers were recorded throughout the F2 crop. Among the five F2 plants, four were gynoecious (100% pistillate flowers) and one plant was monoecious (92.4% pistillate flowers) and from the full sib F2 family one selected plant was gynoecious. These gynoecious plants were pollinated with the pollen from selected monoecious plant and this way the seeds of full sib F3 were collected. The monoecious plant was also selfed creating selfed F3 seed. During summer 2020, all the families (one F3 and four F3 sibs) were raised and observations with respect to proportion of staminate and pistillate flowers were recorded on five randomly selected plants from each population.

Fig. 1. Gynoecious sponge gourd.

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Fig. 2. Gynoecious sponge gourd with flower

RESULTS AND SUMMING UP

Results pertaining to the proportion of pistillate flowers in F2 and F3 generations revealed that like five F2 plants, plants of all the five F3 populations had very high proportion of pistillate flowers, which ranged from 88.0% in line 34/2 to 94.4% in line 19/4. All F3 populations were also characterized by the recovery of at least one absolute gynoecious plant (100% pistillate flowers). In this way we have isolated four gynoecious plants. The advancement of 2-3 generations through selection of gynoecious plants and sib pollinating with plants having a very high proportion of pistillate flowers. Although we have been able to maintain the absolute gynoecious plants through sib-pollination, the detailed genetic study of gynoecism is in progress in order to determine the most appropriate and predictive method(s) of its maintenance through crossing. Thus, it would be imperative to identify suitable molecular markers associated with the sex expression, so that the gynoecious plants can be identified at a very early stage and more efficiently utilized in hybrid seed production. Considering the paramount importance of gynoecious lines in a cost-effective hybrid seed production program, it would also be imperative to develop micro-propagation protocol(s) for its large- scale multiplication and examine its feasibility in hybrid seed production of sponge gourd.

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