Mutation induction of ornamentals *in vitro*

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Abstract

Plant tissue culture is an efficient technique when it comes to plant propagation. The feasibility of combining plant tissue culture with mutagenesis through chemical or physical agent has been tested in our lab. Crops tested include chrysanthemum, *Dieffenbachia*, *Gerbera*, poinsettia and *Kalanchoe*.The higher mutation rates of most chrysanthemums were obtained from callus under γ-ray irradiation at 4 Gy. As well as *Dieffenbachia* was obtained at 1 Gy. The mutation types from compositae flower bud explants had more varieties than those obtained from callus explants, and that replacing γ-ray with NaN_3 as mutagen caused severe decrease in mutagenesis efficiency.The poinsettia hypocotyl explants cut from mature embryos were used and exposed under γ-ray or were subcultured on regenerating medium containing NaN_3. In the end, we found that physical mutagen treatment was more suitable for organgensis and chemical mutagen was more suitable for embryogenesis.lower buds of kalanchoe were exposed to NaN_3, and mutation rates of 77.8 to 53.1% were obtained.

Introduction

Demand for ornamental diversity has always been important in the ornamental market. Creating different flower colors, shapes, while maintaining unified plant type and cultural characteristics contribute to such varieties among ornamental commodities. To this fact, many methods in producing plants with new characteristics have been developed. One of the most commonly used methods is mutagenesis through exposing plants to chemicals (e.g. NaN_3) or radiation (e.g. γ-ray). By treatment of plant cutting under these factors, plant breeders are able to obtain new cultivars far exceeding conventional plant breeding techniques (such as cross breeding… etc). To this date, many ornamental cultivars have already been developed through these techniques and received fruitful results. In spite of its success in plant breeding, however, the high cost in labors, expenses and time of this technique still call for further improvements in its efficiency in propagation and phenotype screening.

Plant tissue culture is an efficient technique when it comes to plant propagation. In addition, it is capable for year-round production and possesses less chimera problems. In view of its advantages, it seems promising that a combination between plant mutagenesis and plant tissue culture may provide a plant breeding technique with greater efficiency and lower cost. The feasibility of combining plant tissue culture with mutagenesis through chemical or physical agent has been tested in our lab. Crops tested include chrysanthemum, *Dieffenbachia*, *Gerbera*, poinsettia and *Kalanchoe*. The following contents address the attempts made and progress received in our lab.

Mutagen on callus culture

Our attempts started off by applying γ-ray irradiation on calli of chrysanthemum and *Dieffenbachia*. Sterilized leaf discs of these crops were cultured in MS agar medium containing with 30 g/L of sucrose, 5 mg/L of IAA, 10 mg/L of BA and 1 mg/L of 2,4-D under pH5.7 to induce callus formation. The culture was conducted under 25±3℃ and 35±5μmolm^{-2}s^{-1} of lightness for 16 hrs photoperiod. Calli were further multiplied in MS medium containing 1 mg/L of BA and 0.5 mg/L of IAA. After 1 week of subculture, the callus explants were exposed under γ-ray radiation (1~4Gy). Following the formation of adventitious shoots and ex vitro of cuttings, plants with extraordinary phenotypes were selected, cloned a grown into cultivars. Results showed that mutation rates of the treated plants differed between different cultivars, with chrysanthemum 'Red Beauty' showing the highest mutation rate. In spite of such differences, the highest mutation rates of most cultivars were obtained under the same dosage of γ-ray irradiation (4Gy).

The same method was also tested on *Dieffenbachia*. In this plant, different mediums was used (1/2MS basal medium containing BA 3 mg/L and NAA 0.25 mg/L, or BA 5 mg/L and NAA 0.125 mg/L, respectively) and the mutation induction was most effective under 1Gy of γ-ray radiation. In the end, many varieties of *Dieffenbachia* were bred successfully.

Mutagen on flower culture

We continued our study on the mutation of spray chrysanthemum and *Gerbera*. Since head flowers (special inflorescence of compositae) are sterilized easier, the efficiency of the combination of flower bud culture with induced mutagenesis in these plants was tested. Our findings indicated that, after mutagenesis induction, efficiency

of developing adventitious bud from spray chrysanthemum and *Gerbera* head flowers differed among different cultivars, with the shoot development stage as the optimal timing for mutagenesis induction, and 4Gy γ-ray irradiation (once or twice) as the optimal dosage. In addition, we found that mutation types from flower bud explants had more varieties than those obtained from callus explants, and that replacing γ-ray with NaN_3 as mutagen caused severe decrease in mutagenesis efficiency.

Mutation via embryogenesis

Another attempt was made on poinsettia according to our previous finding in poinsettia micropropagation that callus explants of poinsettia can proliferate into embryos easily, and the fact that most poinsettia cultivars were developed by mutation. Poinsettia shoot tip were grown on MS medium containing 0.4~0.8 mg/L of 2,4-D and 0.2 mg/L of BA to induce callus, and subcultured on the same medium for callus multiplication. Following the embryogenesis of these calli on MS medium containing NAA 0.2 mg/L and BA 0.2 mg/L, they were exposed under 1~4Gy γ-ray radiation (once or twice). Global embryos formed were used as explants for secondary embryos, which were then, after applying riboflavin in medium, developed into cotyledon embryo. Finally, the hypocotyl explants cut from mature embryos were used and exposed under γ-ray or were subcultured on regenerating medium containing NaN_3. In the end, we found that physical mutagen treatment was more suitable for organgensis and chemical mutagen was more suitable for embryogenesis.

Finally, our most recent study focused on the breeding of *Kalanchoe garambiensis,* an endemic and endangered species in Taiwan, which is known to produce few offspring with low or poor fertility after inter specific crossing. In addition, many species of *Kalanchoe* can also produce plantlets on the leaf margin through embryogenesis. Flower buds of kalanchoe interspecific cultivars ‘Beacon’

and ‘Sunrise’ were exposed to NaN_3, and mutation rates of 77.8 or 53.1% were obtained respectively. Flower colors of these mutants changed from bright orange to oreddish orange, light orange or yellow. Moreover, leaf phenotype changes were also observed in these mutants.

In conclusion, our approach of combining plant mutagenesis with plant tissue culture in ornamental breeding has received fruitful results in chrysanthemum, *Dieffenbachia*, *Gerbera*, poinsettia and *Kalanchoe*. With little modifications in the culture condidiond of these plants, and the correct choice of type and dosage of mutagen, this technique shall provide us an efficient way in ornamental breeding.

Acknowledgements

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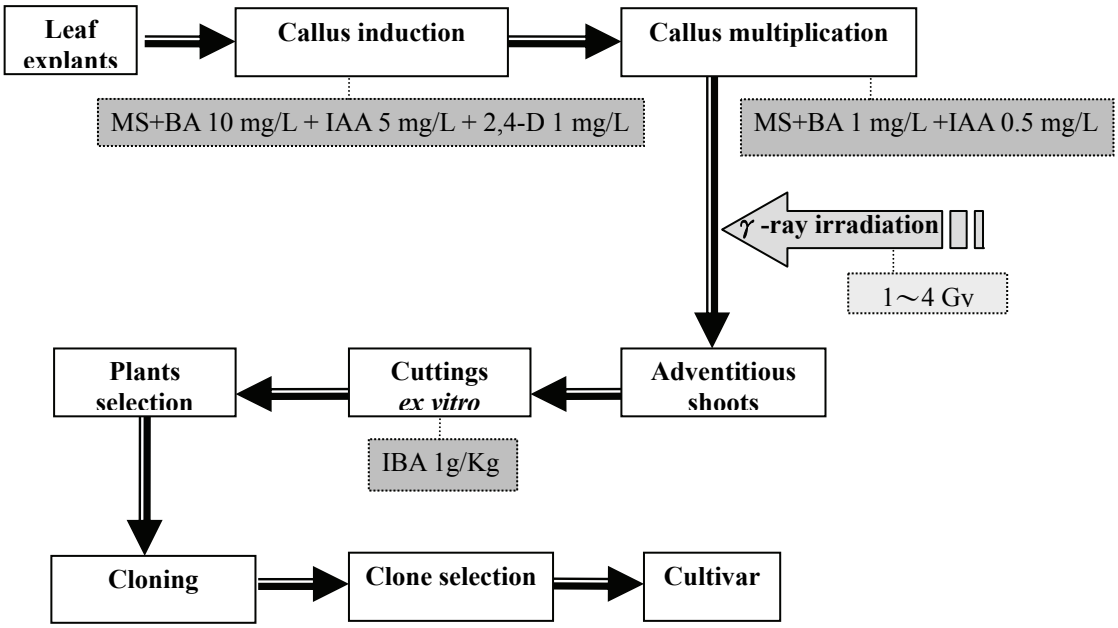

Fig.1. The procedure of chrysanthemum mutagenesis from leaf callus

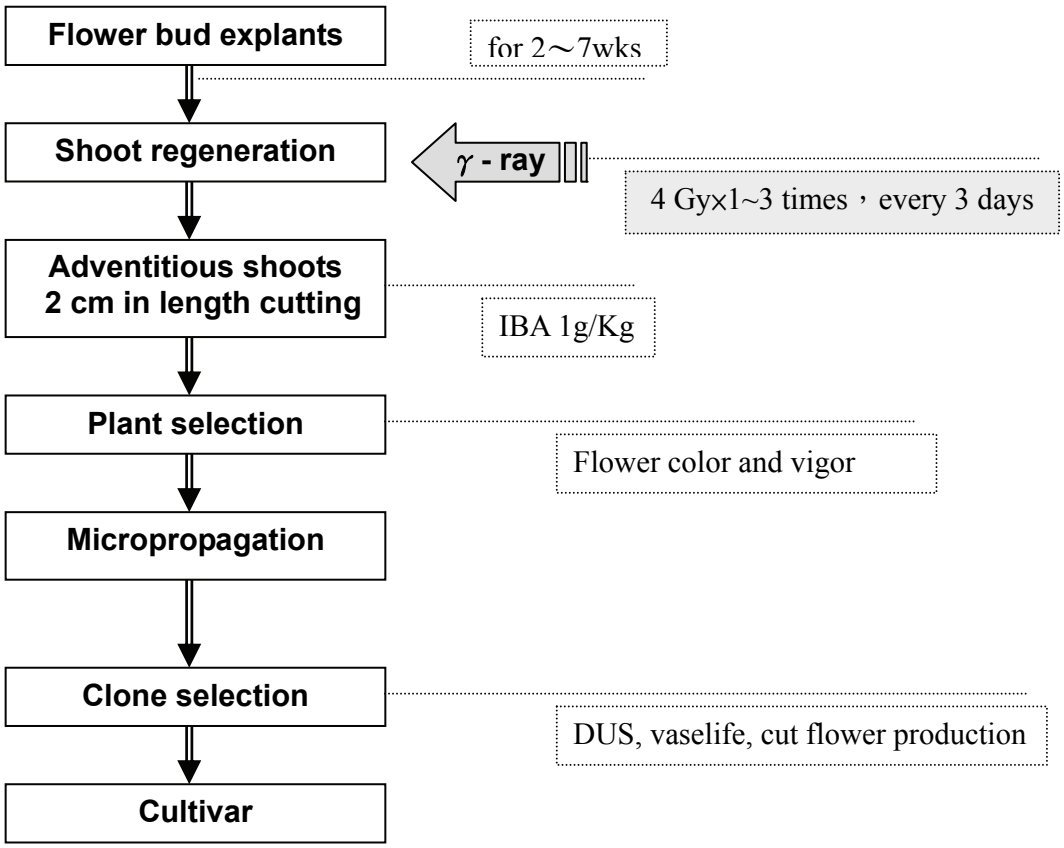

Fig.2. Procedure of chrysanthemum or gerbera mutagenesis from flower buds

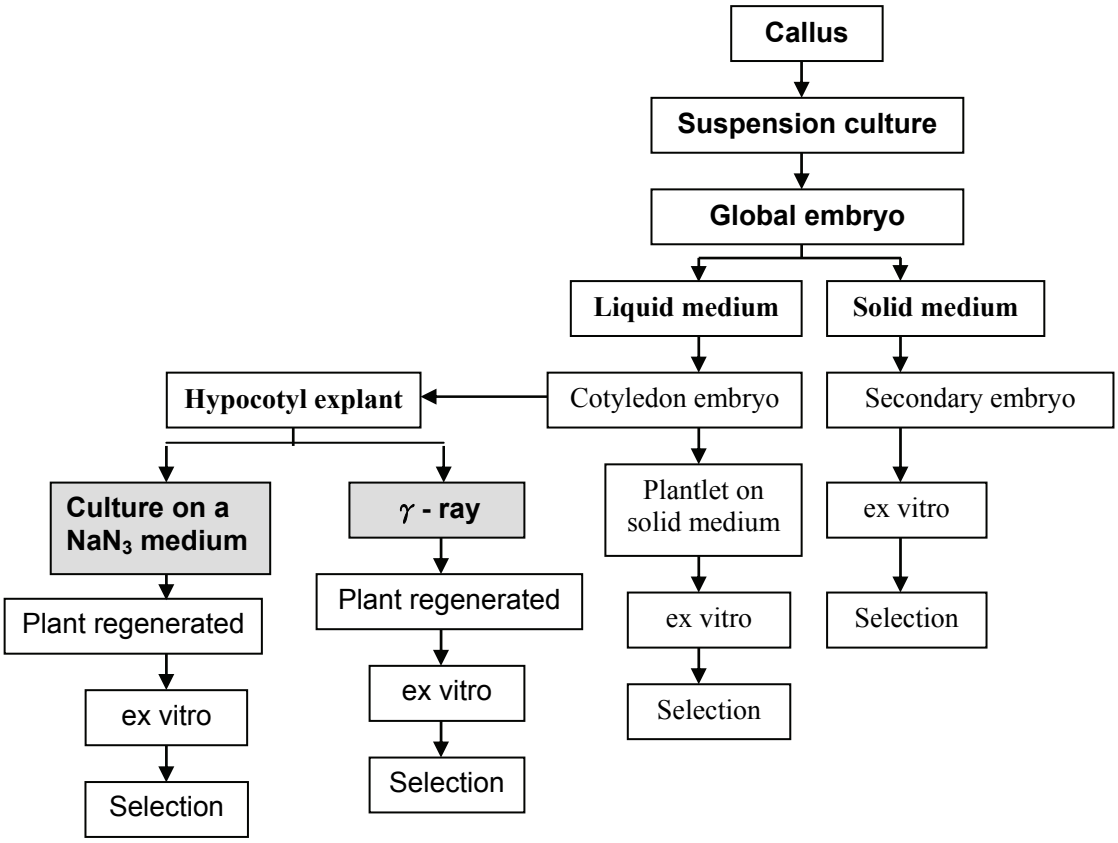

Fig.3. Poinsettia embryogenesis and mutagenesis in liquid medium

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