Note

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Induction of Morphologically Changed Petals from Mutagen-treated Apical Buds of Rose and Plant Regeneration from Varied Petal-derived Calli

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Abstract

The apical buds of lateral branches asexually multiplied by cutting were treated with some chemical mutagens, and the growth and differentiation or morphological changes of the mutagen-treated buds were traced in developed flowers. As a result, the variations in size, shape, color and number of petals were detected most frequently in the flowers that were developed from apical buds treated with N-methyl-N'-nitro-N-nitrosoguanidine at 100 μg ml^{-1}. The variant petals were cultured on MS medium supplemented with NAA and BAP for *in vitro* isolation and multiplication of morphologically altered rose plants. Embryogenic calli were obtained via adventitious roots induced from the petals and successfully differentiated to intact plants. Consequently, the regenerated plants produced the varied flower different from that originally used for tissue culture. Thus, the present study suggested that our approach would provide an effective method for easily and rapidly inducing variations in flowers of rose and for *in vitro* multiplication of their regenerants.

Key words: Rose, chemical mutagenesis, plant regeneration

Because of high commercial values as an ornamental crop, numerous mutants have been induced in rose plants by the use of chemical and physical mutagens. Especially, the irradiation of X- and γ-rays on buds was highly effective for causing mutations in relation to floral characteristics such as flower colors and shapes in flowering roses (Broertjes and van Harten, 1988). In the case of roses, useful variations could be easily availed through vegetative propagation such as cutting, and therefore the mutation breeding is a promising tool for improving cultivars of flowering roses. Nevertheless, the recurrent 'cutting back' for enlarging mutational sectors was a time-, space- and labor-consuming procedures, typical in the conventional breeding process for utilizing bud sport in rose plants. This disadvantageous process has been successfully improved when variegated tissues or organs were *in vitro* cultured for mutant isolation, propagation and plant regeneration (Shigematsu and Matsubara, 1972; Nagatomi, 1991). Also in roses, we have attempted to establish the system for the mutation breeding in combination with an *in vitro* culture of varied tissues or organs, since we have established plant regeneration from leaf-derived (Toyoda *et al.*, 1993) and petal-derived calli (Chatani *et al.*, 1996). The present paper describes the highly frequent occurrence of morphological changes in petals which were developed from mutagen-treated flower buds and the subsequent plant regeneration of variants via calli derived from varied petals.

The cuttings (with two or three leaves) of rose (*Rosa hybrida* cv. Carl Red) were inserted into water-saturated rockwool cubes and grown at 18 ± 4 ℃ for one month for adventitious root formation, and then transplanted to soil in a pot according to standard horticultural procedures (Preece and Read, 1993). The apical buds formed in the lateral branches were used in the following experiment for mutagenesis.

In our cultivation of rose plants, we first clarified the developmental change of lateral buds to flower bud formation in order to determine the most effective timing for the mutagen treatment. For this purpose, we harvested apical buds of lateral

branches at the different foliage stages of rose plants asexually propagated through cuttings and anatomically examined the developmental stage of meristematic tissues in the buds under a dissecting microscope. As a result, the differentiation of primordial flower buds was initially observed in the meristem of the apical buds at the third foliage stage, and at the fifth foliage stage the sepals were well-developed and the petals, pistils and stamens were formed at the inner side of calyx. Judging from these results, the apical buds at the third foliage stage were considered to be the most effective target for mutagen treatment, because of rapid detection of induced variations in petals differentiated directly from mutagen-treated organs.

The chemical mutagens used in the present study were N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), ethyl methane-sulfonate (EMS), azacytidine (AC) and acridine orange (AO). These mutagens are grouped to alkylating agents (MNNG and EMA), base analogues (AC) and intercalating agents (AO) and well-known to be incorporated frequently in actively proliferating tissues such as developing buds (Fehr, 1987). In the present study, 20 μl of the mutagen solution (containing the chemicals at concentrations of 10-200 μg ml^{-1}) supplemented with an aliquot of Tween 20 (0.8 μl ml^{-1}) was dropped onto non-differentiated immature apical buds at the third foliage stage by a syringe, and the growth and differentiation of the mutagen-treated buds were traced everyday.

Table 1 shows varied flowers with abnormal petals developed from mutagen-treated apical buds. Although some apical buds ceased their growth and withered with the present treatment, the flowers carrying variations in size, shape, number and color of petals were successfully developed from mutagen-treated apical buds, and most frequently when treated with MNNG at 100 μg ml^{-1}. As shown in **Fig. 1**, the color of petals was changed from red to pale red (C, D, G, I and K) or pale pink (E, F, H and J). Moreover, petal number was reduced in E, F, G and L, while in I and J the flowers increased in number of petals. The size of the petals became smaller in most cases. Thus, the present treatment of apical buds with MNNG was effective for promptly detecting morphological changes in the differentiated petals and most of the flowers obtained possessed multiple morphological variations in petals.

For isolation and propagation of variants, a tissue culture from abnormal petals was conducted according to the method described previously (Chatani *et al.*, 1996). In the present study, the flower varied in size and color, as shown in **Fig. 2A**, was used for callus induction and plant regeneration. Namely, the receptacles and sepals were removed from the flower, and the remaining petals were excised, surface-sterilized, segmented and cultured on a Murashige-Skoog medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.75 μg ml^{-1} α-naphthaleneacetic acid (NAA) and 0.01 μg ml^{-1} 6-benzylaminopurine (BAP). As a result, the petal explants induced adventitious roots and then produced bugle-like embryogenic calli from the

Table 1. Occurrence of variants with different flowers from originals by mutagen-treatment.

Mutagens used	Concentrations (μg ml^{-1})	Number of mutagen-treated apical buds			
		Total	Withered	Flower-developed	
				Normal	Abnormal*
MNNG	50	50	1	45	4
	100	50	8	30	12
	200	48	9	31	8
EMS	50	49	1	47	1
	100	52	6	41	5
	200	48	3	39	6
AC	50	50	6	44	0
	100	50	2	46	2
	200	50	6	43	1
AO	50	45	5	40	0
	100	50	5	45	0
	200	50	6	43	1

*Flowers including variations singly or multiply in number, color, shape and size of petals.

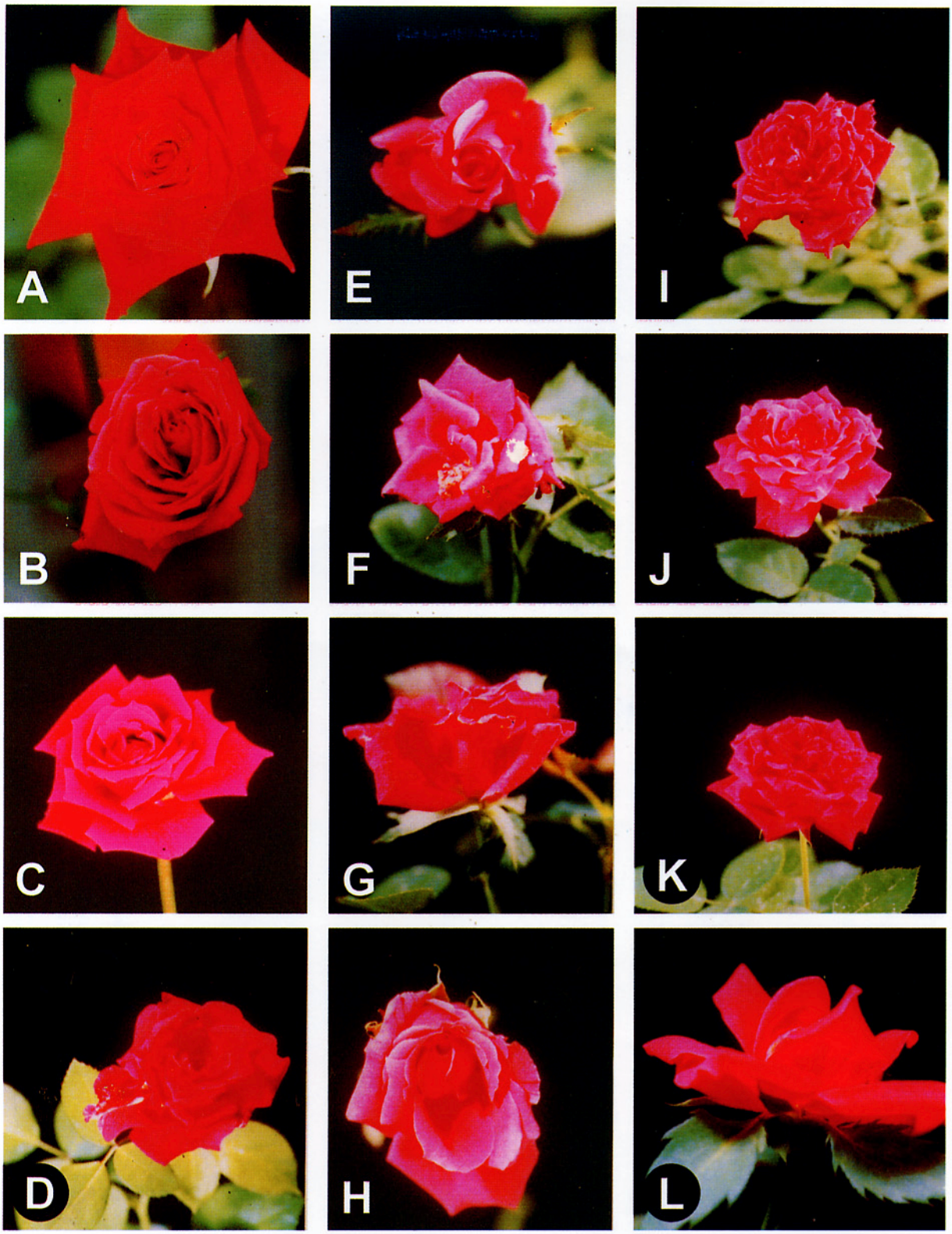

Fig. 1 Flowers with morphological abnormal petals developed from mutagen-treated apical buds of rose. Non-differentiated immature apical buds at the third foliage stage of rose cuttings were treated with N-methyl-N′-nitro-N-nitrosoguanidine (100 μg/ml) and the subsequent differentiation to flower was observed.

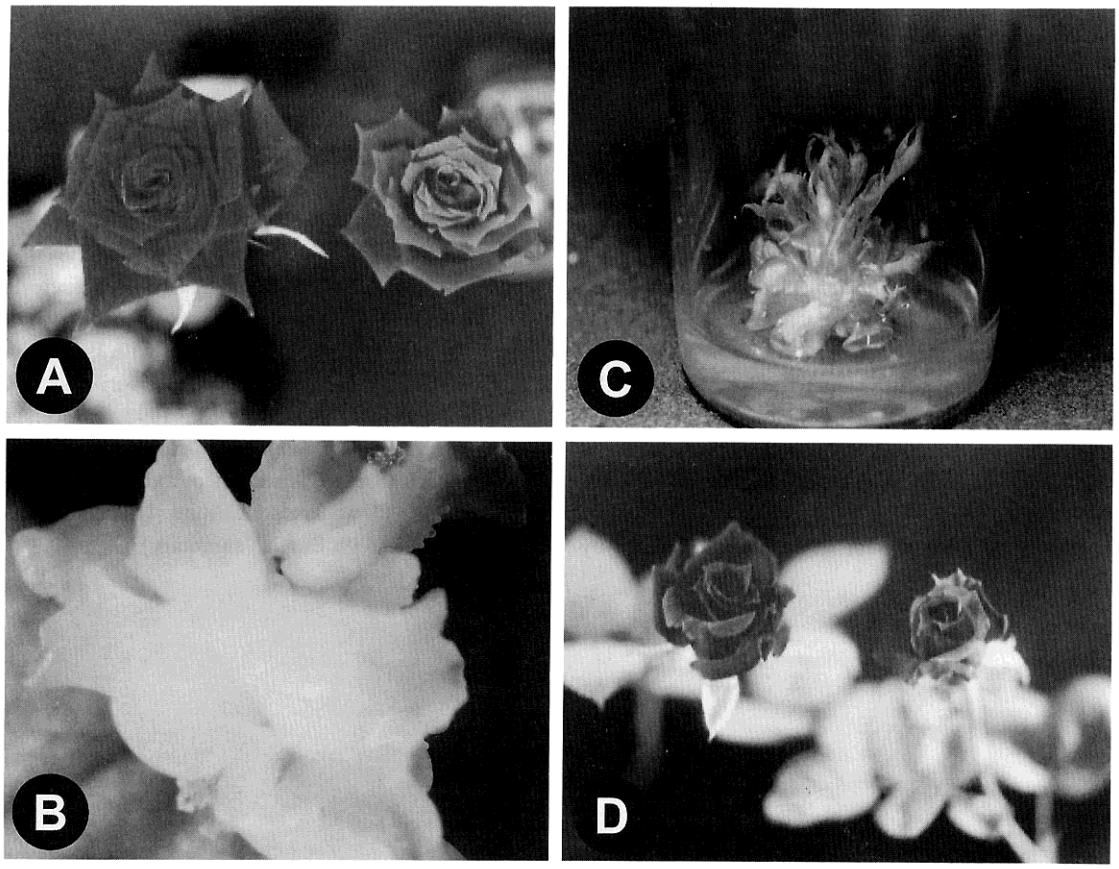

Fig. 2 Tissue culture of morphologically changed petals induced by the treatment of apical buds with MNNG. (A) Normal (left) and varied flowers (right) of Carl Red. The petals were obtained from the varied flower (corresponding to **Fig. 1C**) and cultured on a MS medium supplemented with 0.75 μg ml^{-1} NAA and 0.01 μg ml^{-1} BAP. (B) Bugle-like embryogenic calli induced on adventitious roots which were differentiated from petal explants (two weeks after incubation). (C) Shoots regenerated from embryogenic calli which were transferred to the shoot formation medium (MS medium with 1.0 μg ml^{-1} BAP) (one month after transfer). (D) Appearance of flowers produced by regenerated plants. Of twenty regenerants, 19 plants showed the variant-type flowers (left) similar to the parental one that was differentiated from the varied apical bud and originally used for tissue culture. Only a regenerant produced a malformed flower (right) different from the parental type.

adventitious roots 2 or 3 weeks after incubation (**Fig. 2B**). As pointed out in a previous paper (Toyoda *et al.*, 1993), these calli successfully produced regenerated plants when transferred to the shoot formation medium (MS medium containing 1.0 μg ml^{-1} BAP) (**Fig. 2C**). After acclimation, regenerated plants were transplanted to soil in a pot and tested for their flower formation. In the present experiment, twenty regenerants were successfully obtained from petal-derived embryogenic calli, and almost all of them produced the varied flowers (left in **Fig. 2D**) similar to the shape, size, color and number of the parental varied petals originally used for tissue culture, although only a regenerant produced more malformed flower (right in **Fig. 2D**). Some of the variant regenerants could be asexually multiplied through a cutting procedure under greenhouse conditions. The result indicated that the appearance of flowers in these rose plants was highly constant throughout the repeated cycle of cutting (data not shown), strongly suggesting that the present approach would be an easy and rapid procedure for the mutation breeding of rose, in combination with tissue culture techniques for *in vitro* isolation and propagation of variant plants.

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