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Chemical Suppression of Gravitropic Bending Response in Flower Stalks of Snapdragon (*Antirrhinum majus* L.)

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Abstract : Numbers of chemical agents which have been shown to inhibit either auxin signal transduction pathway or ethylene formation in plant cells were applied to cut flower stems of snapdragon (*Antirrhinum majus* L.) and their effects on the postharvest gravitropic response were studied. The chemical treatments were done by submerging either the stem base or the top part of cut flower, which involves the gravistimulus-sensitive region, for 1 h at 25℃. When the chemicals were supplied from the cut stem base, the gravitropic upward bending of flower stalks kept horizontally after the treatments with 20 mM CDTA or 10 mM $CoCl_2$ was comparable to that of the untreated control, but *o*-vanadate showed a certain degree of effectiveness for suppressing the bending response. In contrast, the direct application of those agents to the gravitropically sensitive region of cut flowers in the presence of 0.01% Triton X-100 resulted in a substantial reduction of the gravitropic response. In the case of 20 mM $CoCl_2$ treatment, almost total elimination of gravitropism without any significant deterioration of flower quality was observed. The results indicate the possibility of preparation of a protocol involving $CoCl_2$ and a proper surfactant for commercial use to suppress the gravitropic response of cut flowers during postharvest storage and transportation.(Received October 16, 1997; accepted November 14, 1997)

Introduction

Gravitropism is a plant response in which asymmetric growth of plant organs stimulated by gravity is involved. When plants are placed horizontally, shoots grow upward (negative gravitropism) while roots redirect their growth downward (positive gravitropism). These negative and positive gravitropic responses are believed to be regulated primarily by auxin.[1,2] Other factors such as Ca^{2+} and ethylene have also been shown to affect the response[2-5]

Essential as it is for the normal plant growth and development, however, gravitropism causes a major postharvest problem in cut flowers. Flowers either with actively growing long spikes such as galdiolus, snapdragon and foxtail lily or with growing peduncles such as anemone and tulip are so sensitive to gravity stimulus that growing regions of these flower stalks bend upward when they are held horizontally during the postharvest storage and transport.[6] In order to prevent the undesired gravitropic bending response, cut flowers are currently stored and transported while being held vertically by using a specially designed container.[6] Therefore, if any chemical treatment method is developed to effectively suppress the postharvest gravitropic response of cut flowers, a considerable cost-down of handling would be expected.

Flower stalks of snapdragon provide a good model system for a study on gravitropism because they show a rapid gravitropic response when placed horizontally. It has been implicated that substances interfering with auxin signal transduction pathway and/or ethylene production in plant cells retard the gravitropic response of flower stalks of snapdragon.[7] In agreement with this, Philosoph-Hadas *et al.*[8] have recently reported that the treatment of snapdragon flower stems with calcium chelators, which modulates cytosolic Ca^{2+} concentration and thus affects signal transduction processes in cells, inhibits the gravitropic response by reducing ethylene production in the bottom portion of the horizontally placed stems. However, the treatment method is yet to be optimized for the commercial application.

Key words : snapdargon(*Antirrhinum majus* L.), gravitropism, cobalt, auxin
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In the present study, examining the effects of some physiological inhibitors such as calcium chelator, P-type ATPase inhibitor and ethylene inhibitor on the postharvest gravitropic response of snapdragon flower stalks, we particularly focused on developing the chemical treatment method which would be commercially acceptable. We herein report that a direct treatment of graviresponding region of the flower stalk with $CoCl_2$, an inhibitor of ethylene production, in the presence of a surfactant for a period as short as 1 h is enough to effectively suppress the gravitropic response.

Materials and Methods

Flower stalks of snapdragon (*Antirrhinum majus* L.) were purchased from market, trimmed to a length of 50 cm and stored in a cold room (4℃) by holding vertically with the stem bases in distilled water. Preliminary experiments showed that the flower stalks kept up to six days at 4℃ were able to normally respond to gravity (Data not shown).

With the stem base put in a test tube filled with distilled water, a flower stalk of snapdragon was held horizontally to initiate the gravitropic response in the dark at 25℃. During the gravitropic response, photos of the flower stalk were taken at every four h in the same frame and then the gravitropic curvatures were measured by the use of a protractor.

Treatments of snapdragon flower stalks with a number of chemicals were performed by two different methods. In the first method, which was practically the same as one employed by Philosoph-Hadas *et al.*,[8] 4 groups of 10 flower stalks were vertically placed in 1 L mass cylinders, each containing 500 *ml* of either 20 mM CDTA, 10 mM $CoCl_2$, 0.5 mM vanadate or distilled water; pH of each medium was adjusted to pH 7 with 1 N HCl or NaOH. After 1 h of the pretreatment, the stalks were retrieved from the treatment solutions and then subjected to gravistimulation in an incubator (25℃) for 24 h. In order to prevent the stems from drying, tap water was occasionally sprayed on stalks of cut flowers and a stainless steel tray filled with tap water was placed on the bottom of the incubator. After the imposition of gravistimulation, portions of the flower stalks showing the gravitropic curvature response were cut and photographed. Curvatures of the flower stalk in the photograph were then measured by using a protractor. The second method was different from the first in that the chemical treatments were done by submerging the upper parts of flower stalks (about 20 cm from the top), instead of the stem bases, in the respective solutions additionally containing Triton X-100 (0.01%). In the case of $CoCl_2$ treatment, the cut flowers were treated with different concentrations of $CoCl_2$ (2-50 mM) for 1 h so as to determine the optimum concentration.

Results and Discussion

Prior to examine the effectiveness of the inhibitors on the gravitropic response of flower stalks, it would be desirable to measure the gravitropic bending kinetics. Several techniques, such as an angular position transducer and the continuous monitoring of bending response by the use of a charge-coupled device (CCD) camera equipped with a computer, have been used to follow the kinetics of stem bending.[9,10] In this study, we followed time-sequentially the gravitropic bending response by photographing a horizontally placed stem at every 4 h during the graviresponse on the same photoframe (Fig. 1a) and then by measuring the degrees of the bending in the photograph by the use of a protractor (Fig. 1b). The gravitropic bending kinetics of snapdragon flower stalks could be described in three phases. In the initial phase, the stalks started to bend at gradually increasing bending degrees. This was followed by a rapid bending in the middle phase, 8 to 12 h after the initiation of gravistimulation. Finally the bending degree was gradually reduced to the end of the gravitropic response. The overall process took approximately 24 h and the stalk eventually bent to 90 degrees in angle. From the photograph shown in Fig. 1a, it appears that the bending response occurred mainly in a certain region of the snapdragon spikes restricted between fully opened flowers and developing flowers.

When snapdragon flower stems supplied with three different chemicals for 1 h from the stem base were gravistimulated for 24 h in the dark at 25℃, a considerable removal of the gravitropic response was observed only in those treated with 0.5 mM *o*-vanadate (Fig. 2). Vanadate is a well known inhibitor of P-type ATPase such as H^+-translocating enzyme located on the plasma membrane of plant cell. Since the proton pump plays an important role in the auxin-induced plant tissue growth as well as in plant gravitropic response, the suppression of the gravitropic response by vanadate treatment is believed to result from blocking a certain step of the auxin signal transduction pathway.[11] Such inhibitory effect of vanadate has previously been noted in oat shoots, where the gravitropic curvature response of the shoot pulvinus segment is completely abolished by

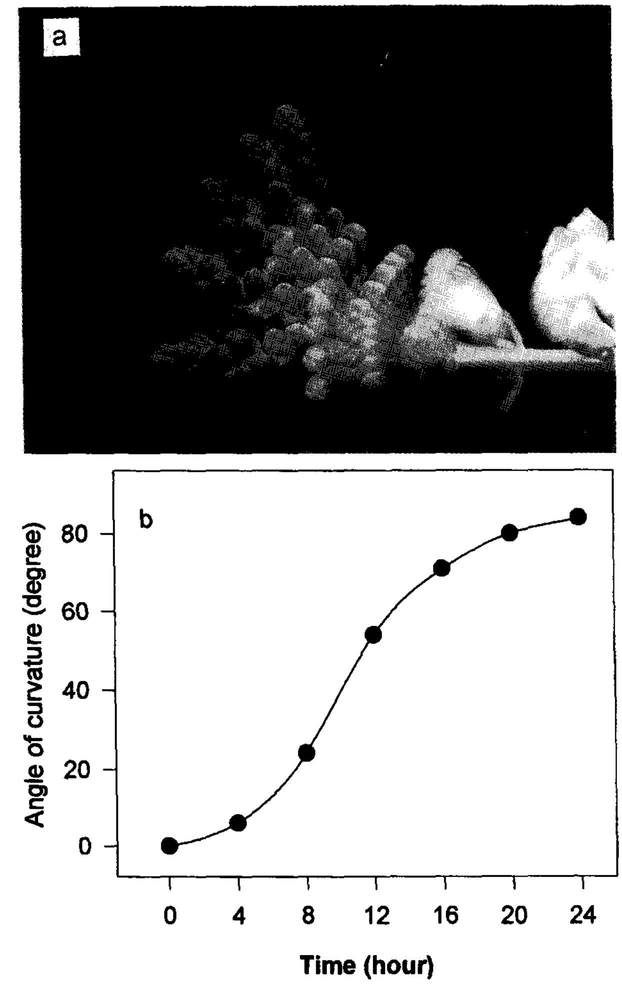

Fig. 1. Photographic illustration of the gravitropic response of cut snapdragon flower (a) and kinetics of curvature (b). Cut flower stem was placed horizontally and photos were taken at every 4 h in the same frame. Bending kinetics was obtained by measuring the curvatures of the stem by using a protractor. Experiments were done three times.

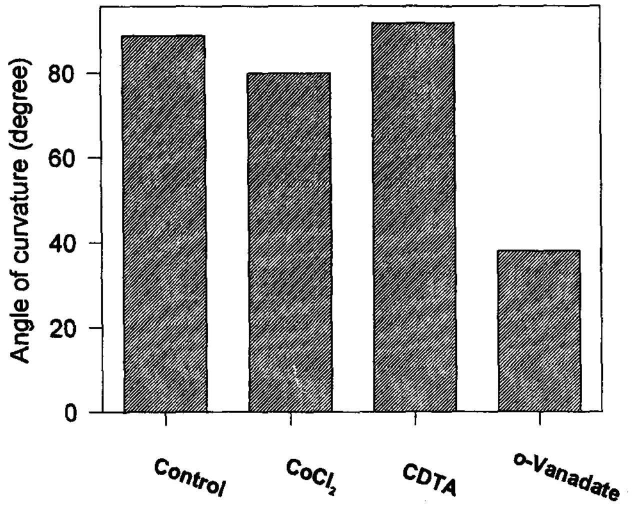

Fig. 2. Effects of pretreatments of cut snapdragon flower stems with chemical agents on the gravitropic bending response. The pretreatments were done at 25°C for 1 h by submerging the stem bases in the solutions (pH 7.0) of either 20 mM CDTA, 10 mM $CoCl_2$, or 0.5 mM *o*-vanadate. The bending angles of 10 flowers per treatment were measured after 24 h of gravistimulation.

the pretreatment of the pulvinus with 0.5 mM vanadate for 12 h.[12] Fig. 2 also shows that both a calcium chelator (CDTA) and an inhibitor of ethylene production ($CoCl_2$) failed to exert the suppressive effect on the gravitropic curvature response of snapdragon flower stems: such seems to be in disagreement with a previous observation made by Philosoph-Hadas *et al.*[8] This discrepancy may arise from the difference in the period of the chemical treatments : note that they treated the flower stems with the inhibitors for 20 h while our treatment was done only for 1 h. Therefore, if the treatment period of the flower stems with CDTA or $CoCl_2$ from the stem base is further extended in order to ensure sufficient amounts of inhibitors being translocated to and accumulated at the graviresponding region of the flower stems, the suppressive effect of those inhibitors would also be expected to be substantiated. From the practical standpoint, however, the extended duration of chemical treatments as long as 20 h was not thought to be commercially acceptable. In this respect, an experimental design was made aiming at shortening the time required for a sufficient accumulation of those inhibitors at the gravitropically active region of the flower stems. For this, we treated the graviresponsive region directly with inhibitors by submerging the top part of the stem in the respective inhibitor solutions containing Triton X-100 (0.01%), a surfactant which was added so as to promote the penetrativity of chemicals into tissues. Fig. 3 shows that the gravitropic bending of cut snapdragon flowers was effectively retarded by 10 mM $CoCl_2$ treatment only for 1 h. Besides, vanadate also showed the suppressive effect to some extent, but the effectiveness of CDTA treatment was only marginal. Such effect may result from the penetration of the inhibitors across the epidermal barrier into the gravitropically growing regions of the flower stem. However, the possibility would not be ruled out that epidermal tissue per se may play an important role in the gravitropic response and thus the inhibitors can act directly on this tissue without penetration, eliminating the gravitropic response. Whichever the case is, it was found that, in cut snapdragon flowers, the undesired gravitropic response can be readily removed by short-term chemical treatments.

We selected $CoCl_2$ as a candidate for the commercial use to suppress the postharvest gravitropic response of cut flowers. In order to determine its optimal con-

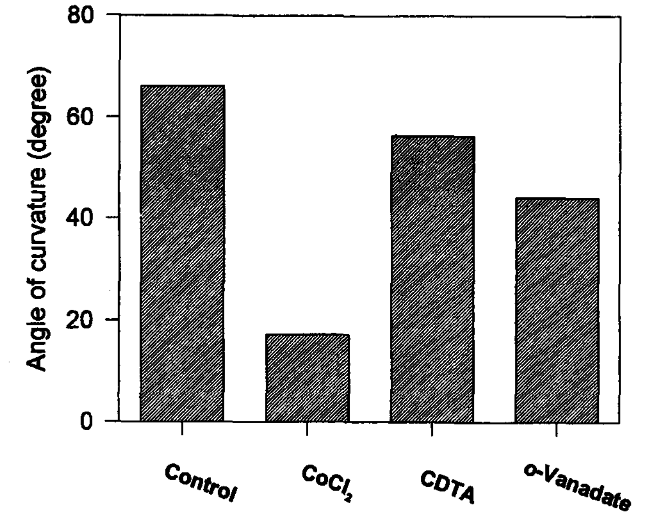

Fig. 3. Effects of direct applications of chemical agents to the gravity-sensitive region of cut snapdragon flower stems on the gravitropic response. For this experiment, top parts of the flower stems were submerged in the solutions (pH 7.0) of the respective chemicals additionally containing Triton X-100 (0.01%). Controls indicate the cut flower whose top parts were submerged in Triton X-100 (0.01%) solution with no other chemicals. The bending angles were measured as in Fig. 2.

centration for the maximum reduction of the gravitropic response without any apparent harmful effects on flower quality, the cut flowers were pretreated with various $CoCl_2$ concentrations by the direct application method of ours. Data shown in Fig. 4 demonstrate that the gravitropic curvature response of harvested snapdragon flower stems decreased with increasing concentrations of $CoCl_2$ in the ranges from 0 to 50 mM and that 20 mM is sufficient enough to completely remove the gravitropic response under our experimental conditions.

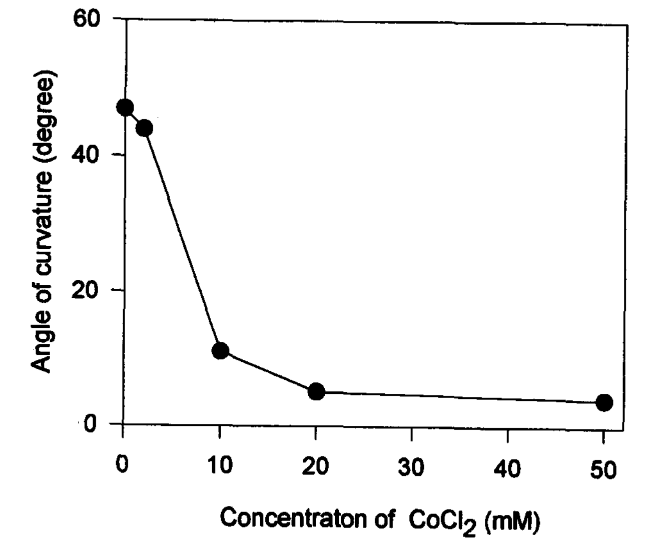

Fig. 4. Concentration dependence of $CoCl_2$ effect on gravitropic response of cut snapdragon flower. Application of $CoCl_2$ and imposition of gravistimulation were the same as in Fig. 3.

Based on the results presented herein, we could tentatively conclude that $CoCl_2$ is an effective and useful agent suppressing the gravitropic response of snapdragon during postharvest storage and transportation, if it is applied directly to the gravity-sensitive region of the flower stem. We are currently conducting a series of experiments in an attempt to optimize the treatment protocol easily accessible for commercial scale applications.

Acknowledgements

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몇가지 생리활성 저해제가 금어초 절화의 굴지성 반응에 미치는 효과

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초 록 : 절화의 수확 후 저장 및 유통과정 중에서 야기될 수 있는 문제 중의 하나인 굴지성 반응에 의한 화경의 구부러짐을 막아내기 위하여 CDTA, $CoCl_2$ 및 ortho-vanadate의 절화 굴지성 반응 억제 효과를 검정하였다. 절화의 굴지성반응억제제 처리는 절화줄기 기부 혹은 굴지성 반응 부위를 시험용액에 담구는 두가지 방법에 의거 실시하였다. 절화기부를 저해제 용액에 1시간 침지시키는 방법으로 처리하였을 경우, P-type ATPase 저해제인 vanadate만 굴지성 반응 억제효과를 보였으나, 절화의 굴지성반응 활성부위를 직접 계면활성제 (Triton X-100, 0.01%)가 녹아있는 저해제 용액에 침지하였을 경우 CDTA 및 vanadate처리구에서도 상당한 정도의 굴지성 억제를 관찰 할 수 있었을 뿐만 아니라 특히 $CoCl_2$ 처리구에서 그 효과가 현저하였다. $CoCl_2$ 농도에 따른 굴지성 반응 억제효과의 변화를 조사한 결과, 20 mM 전후의 농도에서 거의 완벽한 효과를 볼 수 있었다. 이상의 결과를 고려해 볼 때, 적절한 계면활성제와 $CoCl_2$를 이용한 처리액을 준비하여 활성부위에 직접처리하는 방법을 개선한다면 산업적 응용성이 높은 절화의 굴지성 억제 수단을 마련할 수 있다는 결론을 얻었다.

찾는말 : snapdragon(*Antirrhinum majus* L.), gravitropism, cobalt, auxin

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