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Research Note

# Quinclorac, an auxin-type herbicide, induces embryogenic callus and somatic embryogenesis of greater celandine (*Chelidonium majus* L.)

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**Abstract**

Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) is an auxin-type herbicides which was introduced by BASF Aktiengesellschaft in 1985. Quinclorac and 2,4-D applied to embryogenic callus and somatic embryo induction of *Chelidonium majus* tissue cultures. Embryogenic calluses and somatic embryos were not induced from both leaves and stems of *C. majus* using quinclorac and 2,4-D. The root source was only effective for both induction of embryogenic calluses and somatic embryos. Embryogenic callus and somatic embryos were induced from the intact root cultures of *C. majus* using quinclorac. Quinclorac at 0.5 mg $L^{-1}$ was the most suitable concentrations for callus induction and somatic embryogenesis. Quinclorac successfully induced embryogenic callus and somatic embryos from tissue cultures of *C. majus.* In these results, we propose quinclorac as a new plant growth regulator for plant tissue culture.

**Keywords:** *Chelidonium majus*, embryogenic callus, plant regeneration, Quinclorac, somatic embryogenesis.
**Abbreviations:** 2,4-D, 2,4-dichlorophenoxyacetic acid; MS, Murashige and Skoog; Quinclorac, 3,7-dichloro-8-quinolinecarboxylic acid.

## Introduction

Quinclorac is one of quinolinecarboxylic acids (3,7- dichloro-8-quinolinecarboxylic acid) belongs to a new class of highly selective auxin-type herbicides (Grossmann, 1998; Grossmann, 2010; Grossmann and Kwiatkowski, 1995; Wuerzer and Berghaus, 1985). Quinclorac is used in rice and has also been developed for application in turf grass areas, spring wheat, and chemical fallow. The herbicide effectively controls important dicotyledon and monocotyledon weeds, including grass species of *Echinochloa*, *Digitaria*, and *Setaria*, with excellent crop safety (Martinez et al., 1997; Schmidt et al., 1998). Quinclorac controls a broad range of the about 50 *Echinochloa* species and subspecies globally known (Martinez, et al., 1997; Michael, 1981; Schmidt, et al., 1998). However, some of the numerous *Echinochloa* species or biotypes in Southern Europe and the United States are less sensitive to quinclorac (Martinez, et al., 1997). This has been found to be independent of a history of quinclorac application (Martinez, et al., 1997). In Spain, biotypes of *E. crus-galli* and *E. hispidula*, with reduced sensitivity, and a resistant biotype of *E. hispidula* have been described (Martinez, et al., 1997; Martinez and DePrado, 1996; Martinez et al., 1998). The precise mechanism of resistance of these biotypes is not known. Studies suggested that it is not based on differences in uptake, translocation, and metabolism of the herbicide (Martinez, et al., 1997; Martinez and DePrado, 1996). Quinclorac is a systemic herbicide which is readily absorbed by germinating seeds, roots, and leaves and is translocated in the plant both acropetally and basipetally (Berghaus and Wuerzer, 1989; Grossmann, 1998; Lamoureux and Rusness, 1995). Quinclorac showed auxin activity in several auxin bioassays and has been found to act as a synthetic auxin, such as 2,4-D (Berghaus and Wuerzer, 1987). Consequently, quinclorac can be regarded as a new hormone-type herbicide, particularly as quinolinecarboxylic acids with substitution patterns, are thought to be natural plant growth regulators. *Chelidonium majus* (greater celandine) is an herbaceous perennial plant, the only species in the genus *Chelidonium*. It is native to Europe and western Asia and introduced widely in North America (Gilca et al., 2010). The whole plant is toxic in moderate doses as it contains a range of isoquinoline alkaloids but there are numerous therapeutic uses when used at the correct dosage. The main alkaloid present in the herb and root is coptisine. Other alkaloids present include berberine, chelidonine, sanguinarine and chelerythrine (Gu et al., 2010; Sárközi et al., 2006). The effect of the fresh herb is of a mild analgesic, cholagogic, antimicrobial, oncostatic and central nervous system sedative (Ćirić et al., 2008; Lee et al., 2007). A reliable and highly efficient method for the regeneration of intact plants from *in vitro* culture is essential for

**Table 1.** Effect of auxins on the induction frequency of embryogenic callus and somatic embryos from root culture of *Chelidonium majus* after 7 weeks in culture.

| Growth regulators (mg $L^{-1}$) | Embryogenic callus (%)* | Number of somatic embryos/explant** |
|---|---|---|
| 2,4-D 0.1 | - | - |
| 0.5 | - | - |
| 1.0 | 15 | 4.9 ± 0.3 |
| 2.0 | 11 | 4.5 ± 0.4 |
| 4.0 | - | - |
| Quinclorac 0.1 | 18 | 5.3 ± 0.6 |
| 0.5 | 46 | 17.8 ± 1.3 |
| 1.0 | 24 | 7.2 ± 0.5 |
| 2.0 | - | - |
| 40 | - | - |

No response, *From 100 root explants tested.**Values represent the mean ± standard deviation of somatic embryos per explant

establishing a multiple micropropagation and genetic transformation protocol for *C. majus.* The plant regeneration of *C. majus* via somatic embryogenesis using cytokines and auxins has been previously reported. (Kim et al., 1999; Vinterhalter and Vinterhalter, 2002; Woo et al., 1996). In this paper, we introduce quinclorac as a new plant growth regulator for callus induction and somatic embryogenesis in plant tissue culture. We describe the first development of a method for high-frequency somatic embryogenesis and plant regeneration of *C. majus* using quinclorac.

## Materials and methods

### *Seed sterilization and germination*

For preparing plant materials, seeds of *C. majus* were collected from the experimental farm in Chungnam National University (Daejeon, Korea) and stored at 4°C. The seeds were surface-sterilized with 70% (v/v) ethanol for 30 s and 2% (v/v) sodium hypochlorite solution for 10 min, then rinsed three times in sterilized water. Ten seeds were placed on 25 mL of agar-solidified culture medium in Petri dishes (100 × 15 mm). The basal medium consisted of MS (Murashige and Skoog, 1962) salt and vitamin medium solidified with 0.7% (w/v) agar. The MS salt and vitamin medium was adjusted to pH 5.8 before adding the agar and was then sterilized by autoclaving at 121°C for 20 min. The seeds were germinated in a growth chamber at 25°C under standard cool white fluorescent tubes with a flux rate of 35 μmol $s^{-1}$ $m^{-2}$ and a 16-h photoperiod.

### *Induction of embryogenic callus and somatic embryos*

Leaves, stems and roots of *C. majus* were cut into pieces approximately 0.7 × 0.7 cm and 0.7 cm in length, respectively, from plants grown *in vitro* that had been cut aseptically at the ends. Explants were placed on medium (approximately 25 mL) in 100 ×25 mm Petri dishes. Seven explants were cultured in each Petri dish on basal medium consisting of MS medium solidified with 0.7% (w/v) Phytagar (Gibco, Carlsbad, CA) that had been sterilized by autoclaving at 1.1 kg $cm^{-2}$ (121 °C) for 20 min. The pH of the MS medium was adjusted to 5.8 before adding Phytagar. For embryogenic callus and somatic embryo induction from the culture of leaves and stems, the basal medium was supplemented with different concentrations (0.0, 0.1, 0.5, 1.0, and 2.0 mg $L^{-1}$) of quinclora or 2,4-D (2,4-dichlorophenoxyacetic acid). Ten explants were cultured in each Petri dish. For each treatment, 10 Petri plates were sampled and the data combined. Cultures were maintained in a growth chamber in the dark at 25°C.

### *Conversion of somatic embryos and plant regeneration*

Mature somatic embryos were transferred to regeneration media to promote somatic embryo conversion and plant development. The regeneration medium consisted of half strength of MS salts and vitamins. Isolated somatic embryos and regenerated plantlets were incubated at 25 °C in a growth chamber with a 16 h photoperiod under standard cool white fluorescent tubes (35 μmol $s^{-1}$ $m^{-2}$) for 5 weeks.

## Results and discussion

Based on our findings, we believe that a simple and effective protocol has been developed for the *in vitro* somatic embryogenesis and plant regeneration of *C. majus*. We investigated the effects of different concentrations of quinclorac and 2,4-D on the induction frequency of embryogenic calluses and somatic embryos from leaves, stems and roots of *C. majus* after 6 weeks in culture. Embryogenic calluses and somatic embryos were not induced from both leaves and stems of *C. majus* using quinclorac and 2,4-D. The root source was only effective for both induction of embryogenic calluses and somatic embryos. Embryogenic calli and somatic embryos were induced from intact roots of *C. majus* cultured on MS medium supplemented with 0.1–1.0 mg $L^{-1}$ quinclorac or 1.0–2.0 mg $L^{-1}$ 2,4-D. With increasing the concentration of quinclorac, induced both embryogenic callus formation and number of somatic embryos up to the concentration of 1.0 mg $L^{-1}$ and further increasing the quinclorac concentration, embryogenic callus did not form any more. Quinclorc at 0.5 mg $L^{-1}$ was the most suitable concentrations for callus induction and somatic embryogenesis. In this concentration 46% embryogenic callus was formed and 17.8 somatic embryos were developed from each callus (Table 1). On the other hand 2,4-D was also able to induce both embryogenic callus and somatic embryos, but was not as much as quinclorac. Here at the low concentrations (0.1–0.5 mg $L^{-1}$) embryogenic callus did not appear at all but further increasing the concentration of 2,4-D , embryogenic callus and somatic embryos were developed up to the concentration of 2 mg $L^{-1}$ and then further increasing the concentration, no embryogenic callus was formed. The concentration of 2,4-D at 1.0 mg $L^{-1}$ induced 15% embryogenic callus and 4.9 somatic embryos in each callus. The concentration of 2,4-D at 2.0 mg $L^{-1}$ induced slightly lower embryogenic callus and somatic embryos in each callus.Under a microscope, various stages of somatic embryo development could be observed (Fig. 1). The root explant cultured on medium containing 0.5 mg $L^{-1}$ quinclorac developed yellowish embryogenic callus within 2 to 3 weeks.

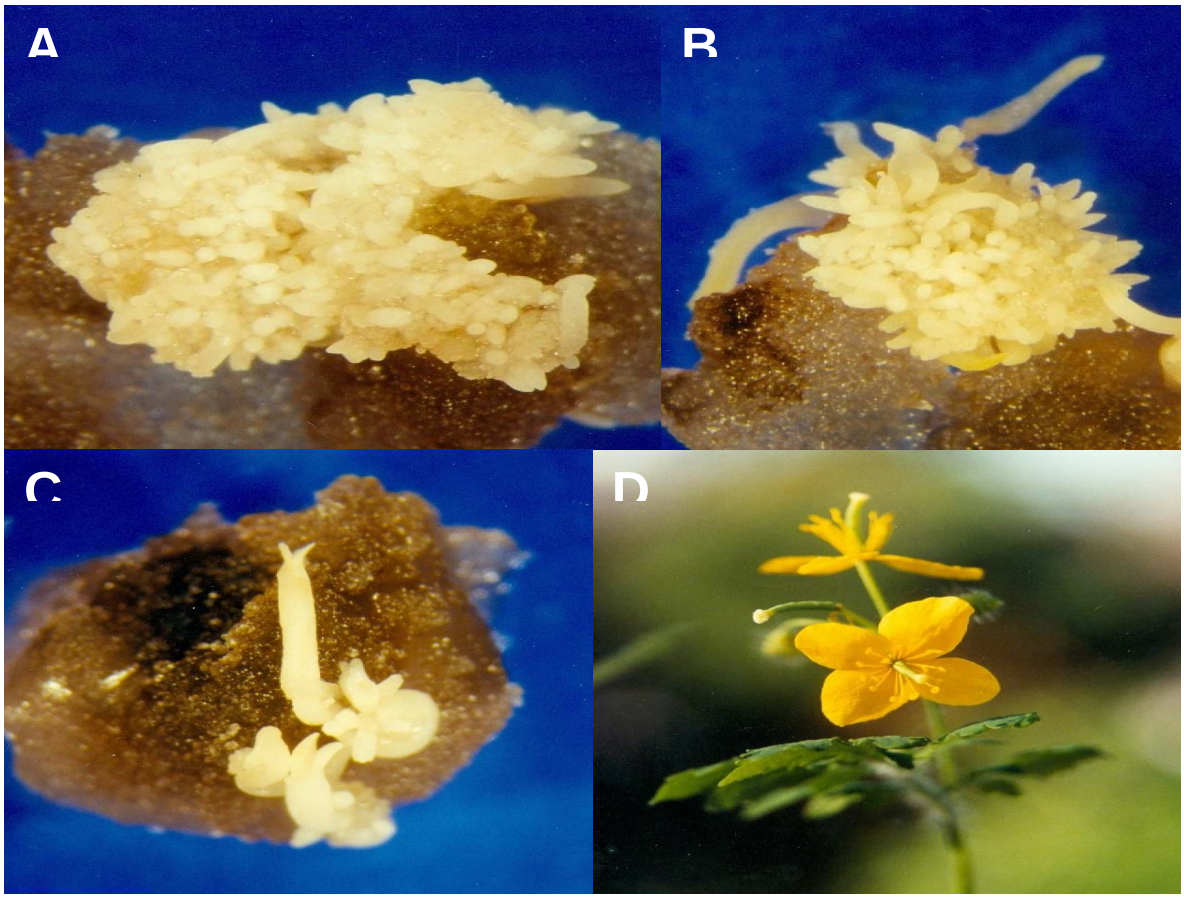


**Fig 1.** Somatic embryogenesis and plant regeneration in *Chelidonium majus* L. Numerous globular, heart (B), torpedo (C), and cotyledonary somatic embryos are shown developing on the surface of embryogenic callus from root cultured on solid MS medium supplemented with 0.5 mg/l quinclorac. Flowering regenerated plant (D) in the green house. Magnification: A–C, x10; D, x1.

Small globular and heart stage somatic embryos (Fig. 1A) were visible within 4 weeks. The establishment of embryogenic cultures was followed by the development of torpedo (Fig. 1B) and cotyledonary (Fig. 1C) stages of somatic embryos within 5 to 7 weeks. Somatic embryos at the cotyledonary stage of development were selected for conversion and shoot growth. Mature somatic embryos were transferred to regeneration media to promote plant development and cultured in growth chamber for 5 weeks. Before transferring regenerated plants to pots, the plantlets were subcultured on half-strength MS salts and vitamins in the absence of growth regulators for 1 month. Plantlets were then transferred to pots containing autoclaved vermiculite, covered with polythene bags to maintain high humidity, and kept at 25°C in a growth chamber for 1 month. After 1 week, the bags were perforated, and the plants were hardened. The plants were transferred to soil with a 82% survival rate where they grew normally and flowered within 3 months (Fig. 1D). Plant tissue culture plays an important role in plant regeneration and micropropagation. The term “regeneration” has been broadly used in the context of tissue culture to refer to the production of whole plants from cells, tissues, organs, meristems, or zygotic embryos cultivated *in vitro*. There are two major systems of plant regeneration: organogenesis and somatic embryogenesis. These systems are defined based on the developmental stages through which a whole plant is regenerated (Phillips and Hubstenberger, 1995). Many plant species can be regenerated and propagated by the production of somatic embryos derived from diverse explant tissues (Ammirato, 1989). In plant tissue culture, several auxin (hormone) type herbicides such as chlorophenoxy acids (e.g., 2,4-D, 2,4,5-T, and MCPA), benzoic acids (e.g., dicamba), and pyridines (e.g., picloram) have been applied to callus induction and somatic embryogenesis. Although 2,4-D is the most common form of exogenous auxin provided to induce somatic embryos from plant cell and tissue cultures, quinclorac has been found to promote somatic embryogenesis in seed-derived zygotic embryo explants of sweet pepper (*Capsicum annuum* L.) (Steinitz et al., 2003). The system described here for the production of *C. majus* somatic embryos and regenerated plants without loss of morphogenetic capacity using quinclorac could be a model for introducing quinclorac as a new plant growth regulator for callus induction and somatic embryogenesis in plant tissue culture.

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