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BRIEF COMMUNICATION

# The effects of electric current on flowering of grafted scions of non-vernalized winter rape

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## Abstract

Non-vernalized scions were grafted onto vernalized stocks in winter rape (*Brassica napus* L. var. *oleifera*, cv. Górczański). The grafted plants were subjected to electric current (30 V for 30 s or 6 V for 24 h) and the percentage of flowering scions was recorded. The negative polarity with cathode (-) attached to the scion and anode (+) left close to the roots inhibited greatly the percentage of flowering. The reverse polarity enhanced flowering markedly under short days and only slightly promoted flowering under long days. Attachment of electrodes without passing a current had no effect on flowering.

*Additonal key words*: *Brassica napus*, photoperiodic induction of flowering, transmission of floral stimuli by grafting; transport of floral signals.

Both enhancing and inhibitory effects of direct electric currents (DC) on flowering have been reported. DC inhibited flowering in the long-day (LD) plant spinach when the cathode was connected to the leaves and the anode to the roots (Montavon and Greppin 1983, 1986). We confirmed such an inhibitory effect of DC on flower induction in the short-day (SD) plant *Chenopodium rubrum (*Adamec *et. al.* 1989). In contrast to these observations, DC of positive polarity (anode at the apex) increased the percentage of reproductive plants in partially vernalized winter wheat (Filek *et al.* 2002).

Winter rape has been chosen as another experimental model to test the effects of DC on flowering. Grafting non-vernalized scions onto vernalized stocks of winter rape causes its reproductive development in the scions (Dubert *et al.* 1988, Filek *et al.* 1988). The aim of the present work was to examine the effect of DC on the development of the non-vernalized scions after grafting onto the vernalized stocks. As further development of most vernalized plants also depends on photoperiod (*e.g.* Napp-Zinn 1953), we tested the effect of DC under both LD and SD photoperiodic conditions.

Winter rape (*Brassica napus* L. *var. oleifera*, cv. Górczański) was grown in a greenhouse under a 16-h photoperiod, with an irradiance (PAR) of 250 μmol $m^{-2}$ $s^{-1}$, and at day/night temperatures of 20/17 °C. After 4 weeks of cultivation, the plants were transferred to growth chambers for the low temperature vernalization treatment (56 d at temperature 5 °C, 10-h photoperiod, and irradiance of 250 μmol $m^{-2}$ $s^{-1}$). Following vernalization, further cultivation was performed in a greenhouse under similar conditions up to the initiation of the first flower buds. At flower bud inititation the apical part of the plant was cut off and replaced by scions from the apical part (about 2 cm long, with only two uppermost leaves left) of plants cultivated for 4 weeks under non-inductive

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*Abbreviations*: DC - direct electric current; LD - long day; SD - short day.
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greenhouse conditions. Graft unions were covered with *Parafilm* tape and the whole graft was enclosed in a transparent polyethylene bag to ensure a high humidity during the following 7 d. All the leaves appearing on the scions and all the lateral shoots appearing on the stocks were systematically removed.

Two days after grafting the plants were treated with DC. In the first experiment the cathode or anode (silver wire, Ø 0.2 mm) was inserted into the apical part of the graft and the second electrode into the soil near the roots. Two time/voltage combinations were used: 30 V/30 s and 6 V/24 h. The percentage of plants with flower buds was recorded in weekly intervals. The experiment was repeated three times; each treatment included 20 - 30 plants.

In the further experimental set the effects of photoperiod and of electrode localization on the flowering of grafted plants were investigated. Up to the completion of grafting, plants were grown and treated as described in the first experiment. Two days after grafting, the plants were transferred to greenhouse conditions under either 8-h or 16-h photoperiods (250 µmol $m^{-2}$ $s^{-1}$, 20/17 °C day/night temperatures). A DC (30 V/30 s) was applied to plants under both photoperiodic regimes. One electrode was inserted into the apical part of the non-vernalized scion and the second electrode was either inserted into the stem of the vernalized stock below the graft union, or into the soil near the roots. DC current was passed on 2nd and 16th day after grafting. Plants without electric treatment but with silver wire introduced into the apical part to compensate for mechanical stimulation and tissue damage, were used as controls. The percentage of plants with flower buds was recorded 60 d after grafting.

Winter rape cultivated under our experimental conditions exhibited an obligatory requirement for vernalization. Plants not receiving the low temperature treatment or those receiving less than 4 weeks of vernalization remained completely vegetative. From 4 weeks, the percentage of flowering plants increased with the duration of the low temperature treatment, reaching a maximum after 8 weeks.

The results from both experiments confirmed our previous results that the flowering stimulus(i) in winter rape can be transmitted by grafting from a vernalized stock to a non-vernalized scion (Dubert *et al.* 1988, Filek *et al.* 1988). Thus, control plants flowered in both experiments (Figs. 1,2) to about 70 %. The failure to achieve full flowering resulted from failure of a proportion of the grafts to grow properly. The first evidence of transmission of flowering stimuli by grafting was obtained by Chaïlakhyan (1937) with photoperiodic plants (*Perilla)* and was interpreted as indicating the existence of "florigen", a flower-inducing hormone. Later the flowering was also induced in non-vernalized plants of the biennial *Hyoscyamus niger* by grafting it onto the SD tobacco (Melchers 1939). It was concluded that a flower-inducing hormone is formed as a result of vernalization in biennial plants; this hormone exists constitutively in annual plants which thus have no vernalization requirement. Melchers (1939) coined the term vernalin for this hypothetical hormone.

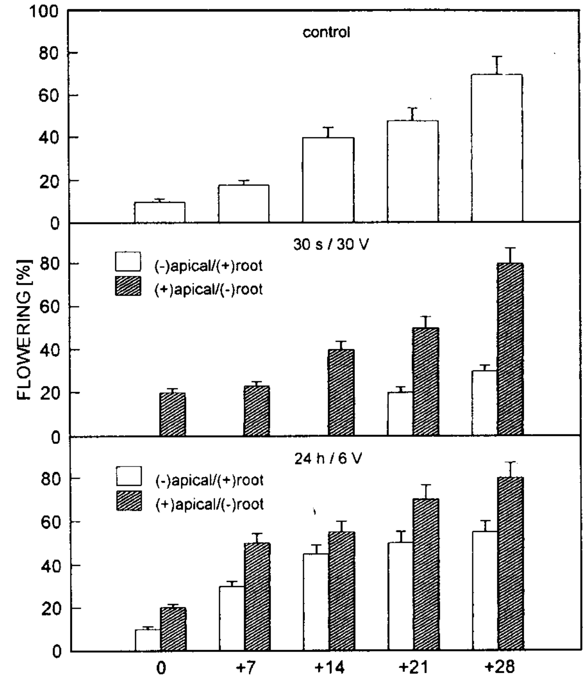

Fig. 1. The percentage of flowering of grafted plants (flower buds observed on non-vernalized scions) of winter rape (*Brassica napus* L. var. *oleifera*, cv. Górczański) recorded in one week intervals after treatment with DC of different voltage, duration and polarity as indicated. Control plants were not subjected to electric current, but electrodes were attached as in treated plants. Plants were kept under 16-h photoperiod.

In both experiments, making the apex electrically negative with respect to the roots inhibited the onset of reproductive development (Figs. 1, 2). None of the plants exposed to DC 30 V/30 s (apex negative) exhibited signs of reproductive development at the second week of observation, compared to 20 % of control plants flowering. Furthermore, only 30 % of treated plants reached the reproductive state at the end of the experiment, compared to 70 % in controls. The inhibitory effect was much weaker with prolonged DC treatment (24 h) at a lower voltage (Fig. 1). Negative polarity inhibited reproductive development in the second experiment (Fig. 2) both under LD and SD. Under SD, when control plants flower to a negligible extent, the inhibition is seen mainly with respect to stimulation of flowering by positive polarity of DC.

The inhibitory effect of an apex-negative DC potential on flowering was observed for the first time by Montavon and Greppin (1983) in the LD plant spinach, and was later confirmed in the SD plant *Chenopodium rubrum* by Adamec *et al.* (1989). In both cases the photoperiodic induction of flowering was prevented. Furthermore, detailed studies of the DC effect, mostly aimed at elucidating its dependence on electrode localization, led us to the conclusion that it was the negative charge anywhere on the path from induced leaves to the apex that was decisive for the inhibitory effect and that DC most likely interferred with the transport of some essential factor(s) for flowering ("floral stimulus"?) (Macháčková *et al.* 1990). DC was also found to interfere with some components of endogenous rhythms involved in flowering in *Chenopodium rubrum* (Krekule and Macháčková 2001).

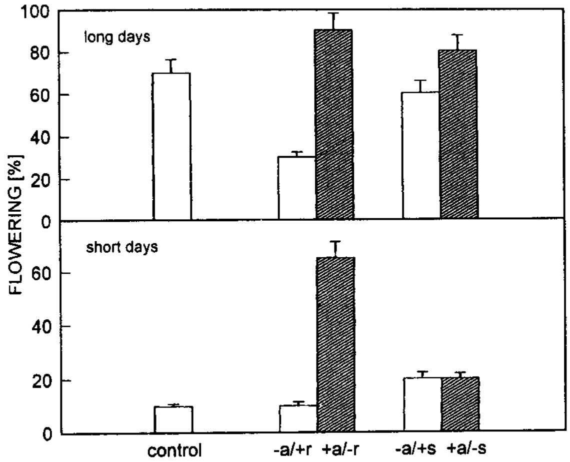

Fig. 2. The percentage of flowering of grafted plants (flower buds observed on non-vernalized scions) of winter rape (*Brassica napus* L. var. *oleifera*, cv. Górczański) treated by DC (30 s/30 V) on 2nd and 16th day after grafting. The plants were grown either on long (16-h) or short (8-h) photoperiod after grafting and one of the electrode was situated at the stem or at the roots as indicated. The evaluation of flowering was carried out 60 d after grafting (a - apical part; r - roots; s - stem; + and – expresses the polarity at the respective organ).

In a general sense, the data reported here for rape are consistent with the above conclusions. However, the negative current did not inhibit flowering in partially vernalized winter wheat and in some cases even a slight stimulatory action was observed as reported by Filek *et al.* (2002). Such inconsistencies may be explained by hitherto unidentified species differences.

Under LD, treatment with DC (scion positive) led to a rise of around 20 % in the percentage of flowering plants compared to controls (Figs. 1,2). Under SD, however, a much greater effect of DC (scion positive) was observed (Fig. 2) and the percentage of flowering plants rose to 75 % compared to 10 % in control plants. Apex positive DC caused an increase in percentage of flowering plants in partially vernalized winter wheat (Filek *et al.* 2002) and thus mimicked additional low temperature exposure. This was in contrast to the finding that positive polarity was ineffective in plants whose transition to reproductive development is determined solely by photoperiod (Adamec *et al.* 1989, Macháčková *et al.* 1990, Montavon and Greppin 1983, 1986). Our results demonstrating the enhancing developmental effect of positive DC in rape are in agreement with those gained in wheat. However, the interpretation that DC generated by an apex positive potential may substitute for low temperature treatment might be misleading for short day conditions in rape. Rather, in this case it is long day induction of flowering which seems to have been substituted by DC. Our data clearly indicate that in winter rape we are dealing with both positive and negative effects of DC.

The comparison of electrode localization close to the roots and at the stem (Fig. 2) has shown that the latter system was much less effective under long days and practically ineffective under SD. This implies a possible role of the roots in the synthesis and/or transport of florigenic signals. However, in our experiments with *Chenopodium,* even the position of electrode on the leaf or at the stem (Macháčková 1990) did not hinder the activity of DC. So far we have no explanation for these differences.

A closer understanding of the phenomena reported in this paper will require a more detailed analysis of the possible interactions between electric currents and the chemical signals involved in the switch from vegetative to reproductive state (phytohormones, saccharides, polyamines, *etc.*), as well as detailed observations of the effects DC treatment on the properties of biological membranes. Experiments to investigate some of these phenomena are already in progress.

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