BIOLOGIA PLANTARUM 50 (3): 465-468, 2006

BRIEF COMMUNICATION

Electric current affects the rate of development in isolated apical parts of rape *in vitro*

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Abstract

Apical parts of stems of *Brassica napus* L. var. *oleifera* cv. Górczański (winter rape) and cv. Mlochowski (spring rape), grown *in vitro,* were subjected to direct electric current (DC) of different polarity, duration and voltage. The positive orientation of DC, *i.e.* anode attached to the apical part and cathode to the medium, markedly enhanced the differentiation of the apical meristem in winter rape. The reverse polarity was without effect. DC treatment of positive polarity resulted in spring rape in transition of all explants to generative state while 70 % of non-treated plants remained at vegetative stage. Even negative orientation of DC brought about a rise in percentage of flowering plants with regard to control. The developmental effects of DC were dependent only to a low degree or not at all on duration and voltage of the treatment.

Additional key words: apical meristem differentiation, flowering factors, vernalization.

Direct electric current (DC) may either enhance or inhibit flowering in plants requiring vernalization or photoperiodic treatment to induce flowering. Both long- and short-day plants are responsive. DC of negative polarity, *i.e.* cathode being connected to the apical part of the plants and anode to the roots mostly inhibits the reproductive development. It was shown in the long-day plant spinach (Montavon and Greppin 1983, 1986), in the short-day plant *Chenopodium rubrum* (Adamec *et al.* 1989) and in winter rape (Filek *et al.* 2003), while the reverse set up, *i.e.* anode at the apical part, increased the percentage of flowering plants in winter wheat (Filek *et al.* 2002) and in winter rape (Filek *et al.* 2003).

The aim of the present work was to study DC effects on transition to reproductive development and use them as a tool for understanding flowering control. We employed isolated apical parts of winter and spring rape *in vitro* and compared the DC effects under conditions when the development is blocked by vernalization requirement in winter rape. Moreover, this system enabled us to better address the role of roots as DC activity was investigated using the system without roots in defined medium.

Seedlings of *Brassica napus* L. var. *oleifera* cvs. Górczański and Mlochowski were grown for 80 d in a controlled environment under a 16-h photoperiod, irradiance of 250 µmol m^{-2} s^{-1} and at day/night temperatures of 20/17 °C. Winter rape was then transferred for 14 d to 5 °C under the same conditions of daylength and irradiance. Such chilling treatment does not completely fulfil the vernalization requirement of this cultivar and the plants further remain at vegetative stage. Following the cold treatment 15 mm long apical segments including the youngest leaf 10 - 20 mm long were cut off from the plants and placed on MS medium (Murashige and Skoog 1962), which contained 0.1 mg dm^{-3} benzylaminopurine (BAP) and 3 % sucrose. The apical parts of spring rape which were not exposed to the

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Received 30 November 2004, *accepted* 21 April 2005.

Abbreviations: BAP - benzylaminopurine, DC - direct electric current

Acknowledgements: This work was carried out in the frame of the Agreement between Polish and Czech Academies of Sciences (project title: Electrophysiological signals in plant development) and supported by a grant to IEB AV0Z5038910.

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cold treatment were collected and further cultivated in the same way. The segments were treated by DC after 2 d of acclimatization. Both polarities were tested. The positive one with the anode (+) attached to the apical part of the stem and the cathode (-) to the medium or to the basal part of the stem and the negative one in a *vice versa* order. The distance between the electrodes was 10 - 12 mm (see scheme in Fig. 1). Different time/voltage combinations were applied: 30 V/10 s, 9 V/30 s, 9 V/1 h, 6 V/24 h. The morphological state of the apical meristem was evaluated after 30 d of growth using a stereomicroscope. The scale of stages of shoot apex differentation was established according to Markowski *et al.* 1986.

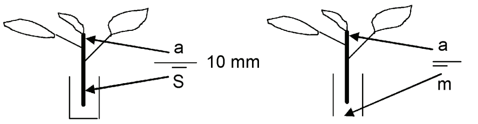

Fig. 1. Schematic view of DC application to plant segments (a - anode, s - stem, m - medium).

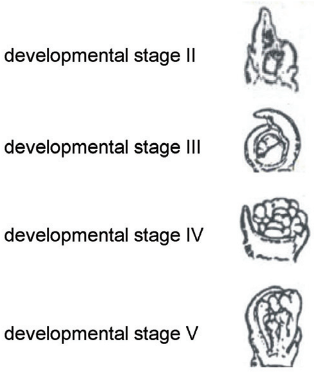

Fig. 2. Developmental stages of the apices of winter and spring rape.

The attachment of anode to the apical part (Fig. 1) enhanced differentiation of the apical meristem. The majority of the explants reached the stage III which is considered as vegetative but indicating further progress towards the generative state (see Fig. 2 for the developmental stages of the apex). There were no differences with regard to the duration and voltage of the treatment (Fig. 3).

The attachment of cathode to the stem instead of medium lowered the positive developmental effect of DC, *i.e.* the percentage of plants reaching stage III as compared to the control, but the effect still remained quite apparent (Fig. 3). The opposite polarity of the treatment, *i.e.* cathode localized at the apex and anode at the medium and/or at the stem, did not bring about any noticeable effect (Fig. 3).

In spring rape nearly 70 % of isolated apical parts of control plants remained vegetative (stage III) after 30 d of *in vitro* cultivation (Fig. 4). The rest reached either the stage IV or V (Fig. 2). The positive DC potential on the apical parts with respect to the medium (Fig. 4) brought about a transition of all explants to the reproductive phase and about 70 % to 80 % of apical meristems were classified as stage V. Again, the positive developmental effect was practically not dependent on the duration and voltage of the DC treatment. This may be clearly demonstrated, *e.g.* when comparing the extreme situations of 9 V/30 s and 6 V/24 h. With the cathode applied to the stem (Fig. 4), the percentage of explants reaching the reproductive state dropped markedly but was still higher than in the control. The opposite orientation of the electric field also resulted in a clear-cut rise of number of generative explants (*ca.* 80 %) as compared to the control and the treatment with the anode localized in the medium was again slightly more efficient then with the anode at the stem (Fig. 4).

Making the apex electrically positive enhanced its differentiation in winter rape and hastened the transition to the reproductive phase in spring rape in our experiments. Such results are in accordance with data gained in winter wheat (Filek *et al.* 2002) and in

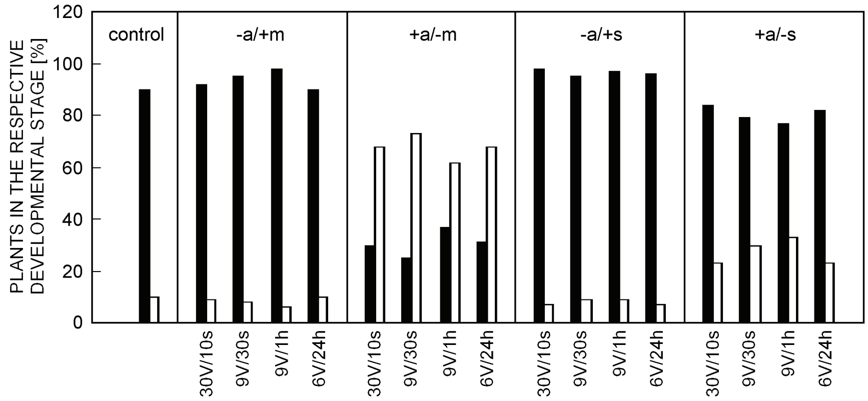

Fig. 3. The percentage of apical meristems of winter rape explants (*Brassica napus* var. *oleifera* cv. Górczański) reaching either stage II or stage III of differentiation (according to Markowski *et al.* 1986, see Fig. 2) after 30 d of *in vitro* cultivation following DC treatment. Voltage and duration of the DC treatment is indicated on abscisa. Polarity and electrode localization: + = anode, - = cathode, a = apical part, m = medium, s = stem. *Black columns* - dev. stage II, *white columns* - dev. stage III (see Fig. 2).

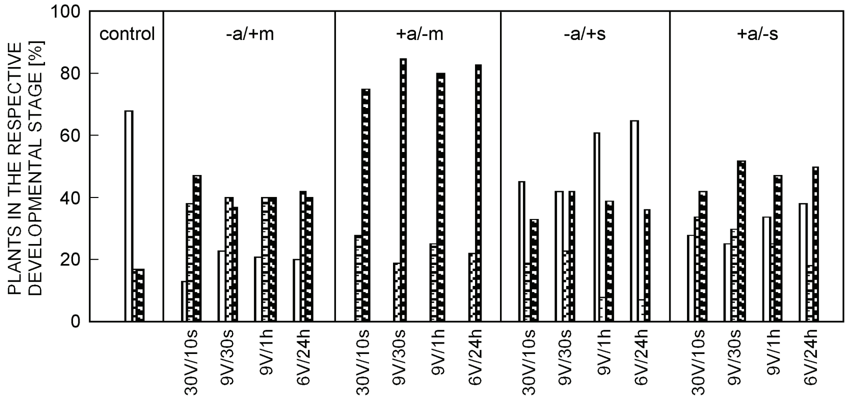

Fig. 4. The percentage of apical meristems of spring rape explants (*Brassica napus* var, *oleifera* cv. Mlochowski) reaching stages III, IV and V of differentiation, respectively (according to Markowski *et al.* 1986, see Fig. 2) after 30 d of *in vitro* following DC treatment. Symbols used as in Fig. 3. *White columns* - dev. stage III, *hatched columns* - dev. stage 4, *black and white columns* - dev. stage V (see Fig. 2).

non-vernalized scions of winter rape (Filek *et al.* 2003), where the positive effect was still accentuated under short days. Formally, the positive polarity thus mimicked the action of low temperatures and long days in our both experiments and in those quoted in the literature. On the other hand the positive polarity was without any effect in the long-day plant spinach (Montavon and Greppin 1986) and in the short-day plant *Chenopodium rubrum* (Macháčková *et al.* 1990). The negative polarity of the apex was without any effects in winter rape but hastened transition to the reproductive state in spring rape. This is in accordance with a slight positive developmental effect of DC in winter wheat as reported by Filek *et al.* (2002). The inhibitory effect of an apex-negative potential on flowering seems to be a more common phenomenon as observed for the first time by Montavon and Greppin (1983) in spinach and confirmed in *Chenopodium rubrum* (Adamec *et al.* 1989) and also by Filek *et al.* 2003 in grafts of winter rape. The photoperiodic induction of flowering was usually cancelled by such effects of DC.

Localization of the electrodes to the medium was always more efficient in affecting development then that to the stem. This was observed in experiments presented here and is in agreement with data on winter rape grafts (Filek *et al.* 2003). This indicates the importance of floral signals coming from the roots and/or the medium.

The general background for the interpretation of the diversity of developmental effects due to DC treatment provides the assumption that they interfere with the transport of florigenic factors indispensable for flowering. These factors are considered in the framework of multifactorial control of flowering (Bernier *et al.* 1993, 2002). It is most likely that they are supplied by both leaves and roots, do have the character of signals and/or flowering factors and contain also inhibitory elements, such as *e.g.* auxin (Krekule *et al.* 1989, Bernier *et al.* 2002). Cytokinins (Macháčková *et al.* 1993, Bernier *et al.* 2002), sucrose, polyamines (Bernier *et al.* 2002) and gibberellins (King *et al.* 2001) are likely candidates for flowering factors. The diversity of DC effects in modifying development is not surprising when taking into account various degrees of polarity found in flowering factors and a variety of cases of flowering control which were investigated and described. It is in this context that pronounced effects of DC polarity were expected and also actually observed. On the contrary we failed to explain the independence of DC action on its duration and voltage as observed in our experiments. However, some common features of the DC effects may reflect common features of flowering control even in different types of inductive mechanisms. The detailed analysis of correlations between changing pattern of transport and distribution of flowering factors and DC developmental effects might become an efficient tool in studying flowering control. Such experiments are in progress in our laboratory.

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