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BRIEF COMMUNICATION

Effect of photoperiod during growth of *Chenopodium rubrum* mother plants on properties of offspring

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Abstract

Using *in vitro* culture, we determined the effect of photoperiod during growth of *Chenopodium rubrum* mother plants on vegetative and reproductive development of offspring. Photoperiod during flowering induction of mother plants (the first 6 d after the germination) has the key influence on seed germination and offspring growth, while offspring flowering and seed maturation is determined by photoperiod their mothers experienced during, and shortly after, flowering induction. The mechanism can be through changes in seed protein pattern which we found dependent on photoperiod experienced by mother plants.

Additional key words: flowering, germination, growth, *in vitro*, maternal effect, seed, seed protein.

Environmental effects on morphological and physiological characteristics of produced seeds (offspring) which took place during development of mother plants, are called maternal environmental effects (Gutterman and Evenari 1972). Plants often respond to environmental changes with phenotypic plasticity and that response may extend to offspring, both by paternal and maternal environmental effects (Galloway 2005). Maternal effects are greater in magnitude, since, in addition to the prezygotic environmental effects, the offspring's early development takes place on the mother plant (Etterson and Galloway 2002). Maternal environmental effects could be elicited by soil nutrients (Straton 1989), irradiance (Galloway 2005), temperature (Lacey *et al.* 1997), photoperiod (Cook 1975, Gutterman 1978, Bertero *et al.* 1999, Munir *et al.* 2001), CO_2 concentration (Steinger *et al.* 2000) and some herbicides (Kern *et al.* 2002). Their expression often depends on the offspring environment, and they are expressed throughout the life cycle of the offspring and may persist for several generations (Amzallag 1999, Galloway 2005). If there is a high positive correlation between successive environments, maternal effect will be advantageous to offspring (Koller 1962). Maternal effects may complicate studies as well as the interpretation of the results (Roach and Wulff 1987). However, the background of those physiological processes is unknown.

Chenopodium rubrum L. is a short day weedy annual, sensitive to the small changes in day length, with defined critical night length of 8 h (Tsuchiya and Ishiguri 1981). It is sensitive to photoperiodic stimulus for flowering as early as at cotyledonary stage (Seidlová and Opatrná 1978), when 6 adequate photoperiodic cycles are sufficient for flower induction. Under the suitable photoperiodic conditions *in vitro,* plant flowers in 15 d (Živanović *et al.* 1995), and produces seeds in 10 weeks (Mitrović *et al.* 2007). We defined optimum media composition for *C. rubrum* growth and flowering (Živanović *et al.* 1995, Mitrović *et al.* 2003), the effect of seed aging on seed germination and seedling growth and flowering (Mitrović *et al.* 2005), as well as the maternal effect of photoperiod on seed number and mass (Mitrović *et al.* 2002, 2007), germination and offspring growth until flowering *in vitro* (Mitrović *et al.* 2002).

Seed structure is quite uniform for family *Chenopodiaceae*, and seed storage proteins are localized mostly in endosperm and embryo (Prego *et al.* 1998). Analysis of *C. incanum* seeds proteins from plants of

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Abbreviations: MS - Murashige and Skoog; PAGE - polyacrylamide gel electrophoresis.
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20 different populations showed more similarities between protein bands in seed samples collected from nearby geographic localities than those from widely separated ones (Crawford 1974).

The aim of this study was to evaluate the effects of maternal photoperiod on seed germination, and offspring vegetative and reproductive development and to evaluate the potential background of those physiological processes on seed protein level.

Chenopodium rubrum L. Sel 184 mother plants were grown from seeds, collected from plants grown from February to May under 10 - 13-h photoperiod in greenhouse. Four days old seedlings were grown *in vitro* on Murashige and Skoog (1962; MS) medium and exposed to 4 different photoperiodic treatments during 10 weeks: 14-h photoperiod, 16-h photoperiod, 6 d of 8-h + 9 weeks of 16-h photoperiod, or 6 d of 14-h + 9 weeks of 16-h photoperiod (Mitrović *et al.* 2007). Those seeds were used for determination of seed protein content, for separation of seed proteins on sodium dodecylsulfate polyacrylamide gel electrophoresis (PAGE), and for testing the effect of maternal photoperiod on germination, offspring growth, flowering and seed development.

Samples of 0.03 g of dry seeds were imbibed 2.5 h in darkness at 32 °C, and powdered in liquid nitrogen. Proteins were extracted for 30 min at 4 ºC with 0.5 cm^3 0.05 M Tris buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA, and centrifuged (4 ºC, 10000 *g* 10 min). Seed protein content was determined by Bradford (1976) method with bovine serum albumin as the standard. All protein measurements were repeated four times.

Separation of seed proteins was performed on sodium dodecylsulfate (SDS) PAGE (Laemmli 1970) carried out under denaturing conditions in gels containing 10 % polyacrylamide with a 4 % stacking gel. A constant current of 25 mA per gel was applied. Proteins were visualized by Coomassie blue staining. All samples were run in triplicate. Relative values of seed protein band intensities were determined in *Image Master Totallab 1.11*.

Seeds were surface sterilized (Mitrović *et al.* 2007). Germination was tested during 4 d (24 h dark at 32 °C, followed by 24 h dark at 10 °C and 48 h white light at 32 °C) in Petri dishes (5 cm^3 sterilized distilled water). Every 24 h, 4 replicates of 100 seeds per treatment were scored for germination. As a criterion of germination, radical protrusion by more than 2 mm was used.

Seedlings with fully developed cotyledons (4-d-old) were transferred to the glass jars containing 100 cm^3 of MS mineral solution supplemented with sucrose (5 %) and gelled with agar (0.7 %), 12 seedlings per jar, and 48 seedlings per treatment of mother plant. They were all exposed to equal 14-h photoperiod for 10 weeks. Irradiance was about 70 $\mu mol\ m^{-2}\ s^{-1}$, provided by 4 fluorescent tubes (18 W, *Osram*, Munich, Germany). Temperature in growth chambers was 25 ± 2 ºC. Height of plants and number of plants with fully developed flowers were determined after 2, 5, 7 and 10 weeks, matured seeds were counted.

Statistical analysis of data was performed using *ANOVA* test, at the 0.05 level of significance.

The difference of just 2 h in maternal photoperiod affects seed germination (Table 1). 35 % of seeds developed and matured under 16-h photoperiod germinated 48 h after the start of imbibition, compared to 24 % of those that developed and matured under 14-h photoperiod. Also significant difference in germination percentage was noticeable among seeds, if their mother plants were grown under different photoperiods during only the first 6 d after the germination, even if equal photoperiod was applied for the following 9 weeks (Table 1). 22 or 48 % of seeds obtained from mother plants grown for first 6 d under 14-h or 8-h photoperiod and than transferred to 16-h photoperiod for remaining 9 weeks germinated 2 d after the start of imbibition, respectively, compared to 35 % of seeds obtained from mother plants grown under 16-h photoperiod for all 10 weeks. On the other hand, seeds from plants grown under equal photoperiod for only first 6 d, and different photoperiods for the remaining 9 weeks germinated in the same percentage. Seeds from mother plants grown for all 10 weeks under 14-h photoperiod and from plants grown for only the first 6 d under 14-h photoperiod and under 16-h photoperiod for remaining 9 weeks, germinated in the same percentage (22 %) (Table 1). Since 6 adequate photoperiodic cycles are sufficient for *C. rubrum* flowering induction, our data points out the key importance of the photoperiod applied during the flowering induction of mother plants on germination of produced seeds. This is in agreement with Cook (1975), who showed the importance of photoperiod during

Table 1. Maternal effect of different photoperiods on seed germination, offspring growth, flowering and number of matured seeds per offspring plant. Offspring plants were all grown 10 weeks under 14-h photoperiod; means ± SE; *n* = 400 (germination) or 48.

Maternal photoperiod	Germination [%]		Height [mm]		Flowering [%]		Seed number
	2nd day	4th day	2nd week	10th week	2nd week	5th week	[$plant^{-1}$]
10 weeks of 14-h	24.0 ± 6.7	87.3 ± 5.1	7.56 ± 0.42	41.82 ± 2.41	34	100	7.50
10 weeks of 16-h	35.0 ± 1.0	95.0 ± 2.6	12.62 ± 2.29	22.28 ± 1.34	55	100	3.35
6 d of 8-h + 9 weeks of 16-h	48.0 ± 1.4	91.0 ± 1.3	8.53 ± 0.19	19.75 ± 0.98	100	100	1.60
6 d of 14-h +9 weeks of 16-h	25.0 ± 2.9	91.5 ± 1.3	10.40 ± 0.32	37.59 ± 1.82	50	100	0.39

C. rubrum flowering induction and shortly after on seed mass.

It was shown that pre- and post-flowering photoperiod regulates vegetative growth (Han *et al.* 2006, Mitrović *et al.* 2007). The effect of maternal photoperiod also affects the offspring growth especially at later stages of development (10th week) (Table 1). Offspring of mother plants grown under 14-h photoperiod are higher compared to offspring of mother plants grown under other three photoperiods (Table 1) and contemporary about 3 times higher (10th week) compared to their mothers (Mitrović *et al.* 2007). Significant difference in stem growth is noticeable among offspring from mother plants grown under different photoperiods for only first 6 d (and under the same photoperiod for following 9 weeks). Offspring of mother plants grown during first 6 d under 14-h photoperiod (and under 16-h photoperiod for the following 9 weeks) and those grown under 14-h photoperiod for all 10 weeks, showed similar stem growth (Table 1). Offspring of mother plants grown under 16-h photoperiod for all 10 weeks are about twice lower. Similar effect of maternal photoperiod was registered on offspring number of leaves (data not shown). Photoperiod to which mother plants were exposed during the first 6 d after the germination (time when they are able to receive photoperiodic flowering induction) left the major effect on plant height, *i.e.* mother plants registered in their seeds information about the photoperiod they had experienced during flowering induction.

The effect of maternal photoperiod on offspring flowering was opposite compared to its effect on growth (Table 1). This could be expected, since transient inhibition of growth occurs at the time of *C. rubrum* flowering (Opatrná *et al.* 1980, Ullmann *et al.* 1980) and growth is inhibited under inductive compared to noninductive photoperiodic conditions (Mitrović *et al.* 2007). Additionally, flowering occurs earlier in limiting environmental conditions, in line with ensuring progeny. Offspring of mother plants grown 6 d under 14-h photoperiod and 9 weeks under 16-h photoperiod or all 10 weeks under 16-h photoperiod flowered in similar percentage (50 or 54 %, respectively), which differed from percentage of flowering offspring from mother plants grown for all 10 weeks under 14-h photoperiod (Table 1). However, 100 % offspring of mother plants grown 6 d under 8-h photoperiod + 9 weeks 16-h photoperiod flowered. Maternal environmental effects on offspring flowering was also noticed in *Plantago major* (maternal soil nutrient effect) and persisted for three generations (Miao *et al.* 1991), and in *Plantago lanceolata* (maternal effect of high temperatures) (Lacey and Herr 2000).

The offspring plants grown under the same photoperiod as mother plants did not show the difference in flowering compared to their mothers (Table 1; Mitrović *et al.* 2007). Offspring of mother plants that have never experienced 14-h photoperiod flowered earlier and in higher percentage than those exposed to 14-h photoperiod.

Maternal effect of different photoperiods also affected number of matured seeds per offspring plant, meaning that maternal effect of photoperiod persist to the second generation, which is in agreement with earlier reviewed data that maternal environmental effects can persist for several generations (Roach and Wulff 1987). The highest number of seeds matured on offspring of mother plants grown under 14-h photoperiod, being, on the same time, about 3 times higher compared to number of seeds collected from their mother plants (Mitrović *et al.* 2007). The lowest number of seeds matured on offspring of mother plants grown under 16-h photoperiod, being many times lower compared to total number of seeds matured on their mother plants (Mitrović *et al.* 2007). In other words, maternal effect of photoperiod is visible in the capability of offspring, grown under the same photoperiod as mother plants, to develop maximum seeds. Maternal effect is the advantage to offspring grown in the same environment as mother plant (Koller 1962, Galloway 2005).

On the basis of our data, arguing that *C. rubrum* mother plants leave the information about photoperiods they experienced in their seeds, we supposed that the background of this information could be visible in composition of seed proteins.

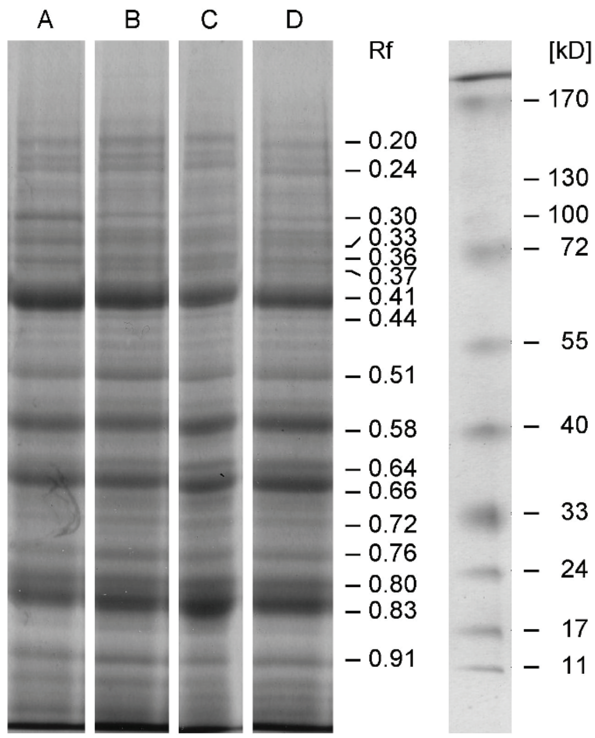

Fig. 1. Coomassie blue-stained 10 % SDS-PAGE gel of *C. rubrum* seed proteins and standard protein molecular mass marker. Marked Rf values represent seed protein bands for which the difference in width and intensity were noticed in seed samples collected from mother plants grown under different photoperiods: 10 weeks of 14-h photoperiod (*A*), 10 weeks of 16-h photoperiod (*B*), 6 d of 8-h + 9 weeks of 16-h photoperiod (*C*), or 6 d of 14-h + 9 weeks of 16-h photoperiod (*D*).

Total protein contents in seeds collected from mother plants grown under 4 different photoperiods showed no significant difference (data not shown). After SDS-PAGE electrophoresis, 33 protein bands were visible (Fig. 1) in all 4 seed samples. For 17 of them, significant differences in width and intensity, were noticed. For 12 bands, high correlations were found between relative intensities and the day lengths mother plants were exposed to, or intensities with seed germination percentage. At 4 bands simultaneous correlations with day length during flowering induction of mother plants and germination percentage, were found. Relative intensities of seed protein bands with Rf values 0.44, 0.58, 0.66 and 0.76 are highly correlated (r = -0.92, 0.87, 0.87, and -0.86, respectively) with the day length mother plants experienced during flowering induction, Rf 0.58, 0.64 and 0.66 (r = 0.96, 0.84 and 0.95) with the day length mother plants experienced during flowering induction and evocation of flowering, and Rf 0.30, 0.36, 0.37, 0.41 and 0.83 (r = -0.99, -0.95, 0.88, -0.91 and 0.85) with the day length mother plants experienced after the flowering induction. This data point out that relative seed protein composition represents an "archive" of photoperiod experienced by mother plants during their lives.

Seed proteins (Rf 0.44, 0.51, 0.76 and 0.91), showing high correlations (r = 0.85, 0.94, 0.97 and 0.99) with germination percentage (2^{nd} day), could be involved in regulation of seed germination. Germination is the first process in plant ontogenesis, so it could be crucial that its regulation is enabled by the information originated from the seed, at the time when the seedling is still not capable to compare its own experience of environment with the information from mother plant (Roach and Wulff 1987). Mazzaella *et al.* (2005) showed that *Arabidopsis* seeds, can compare the information from the environment and those from mother plant left in the seed, mostly by phy B, as early as 5 d after imbibition. High correlations of relative intensities of seed protein bands Rf 0.44 and 0.76 contemporary with both, day length during mother plants flowering induction and germination percentage suggest that seed germination is influenced by photoperiod mother plants experienced during flowering induction.

SDS-PAGE seed protein separation in different species of genus *Chenopodium* showed the difference in width and intensity of bands, but also presence or absence of some protein bands, not only in seed samples of different species, but also in seed samples of the same species collected from widely separated localities (Bhargava *et al*. 2005).

Presence of all 33 protein bands in all 4 samples of *C. rubrum* seeds could be the confirmation that the difference in quantities of some seed proteins is only the result of different photoperiods mother plants were exposed to during their life cycle *in vitro*.

Our results provide a physiological evidences of maternal effect of photoperiod on *C. rubrum* vegetative and reproductive development. The effect is recorded in the seed proteins. It will be of interest to identify relevant protein bands.

In conclusion, maternal effects of photoperiod in *C. rubrum* persist to the second generation. Mother plants relay in their seeds a "protein message" about the day lengths they experienced during their life cycle. Maternal effect of photoperiod extends through the whole life cycle of offspring: photoperiod during flowering induction of mother plants has the key influence on seed germination and offspring growth, while offspring flowering and seed maturation is determined by photoperiod their mothers experienced during induction and evocation of flowering.

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