BIOLOGICA NYSSANA
6 (1) • September 2015: 11-16

Original Article

Received: 28 July 2015
Revised: 28 August 2015
Accepted: 20 September 2015

Maternal effect of continuous light on seed properties in a short day plant *Chenopodium rubrum* L. (Chenopodiaceae)

Aleksandra Mitrović*[*], *Jelena Bogdanović Pristov

Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, Belgrade, Serbia

** E-mail: mita@imsi.rs*

***Abstract*:**

Mitrović, A., Bogdanović Pristov, J.: *Maternal effect of continuous light on seed properties in a short day plant Chenopodium rubrum L. (Chenopodiaceae). Biologica Nyssana, 6 (1), September 2015: 11-16.*

Environmental effects on morphological and physiological properties of offspring which occurs during development of mother plant are called maternal environmental effects. Photoperiod is one of the crucial environmental factors according to which plants modify numerous physiological processes. Maternal effect of photoperiod in a short day plant *Chenopodium rubrum* extends through the whole life cycle of offspring and persist to the second generation, photoperiod during induction and evocation of flowering of mother plants showing the key influence. Here we show that also non-inductive photoperiod preceding flowering induction of mother plants shows its maternal effect on offspring properties: seed size, seed germination and seed protein composition. Presented data argues in favor of earlier suggested that relative amounts of seed proteins represent an "archive" of photoperiods experienced by mother plants during their lives.

Key words: *Chenopodium rubrum*, maternal effect of photoperiod, seed proteins, seed weight, seed germination

***Apstrakt*:**

Mitrović, A., Bogdanović Pristov, J.: *Materinski efekat neprekidne svetlosti na karakteristike semena kratkodnevne biljke Chenopodium rubrum L. (Chenopodiaceae). Biologica Nyssana, 6 (1), Septembar 2015: 11-16.*

Faktori spoljašnje sredine koji utiču na morfološke i fiziološke karakteristike potomstva nazivaju se materinski efekti spoljašnje sredine. Fotoperiod je jedan od ključnih faktora spoljašnje sredine prema kojem biljke modifikuju mnogobrojne fiziološke procese. Materinski efekat fotoperioda proteže se kroz čitav životni ciklus potomstva i održava se i u sledećoj generaciji, pri čemu ključni uticaj ima fotoperiod kome su majke biljke bile izložene tokom indukcije i evokacije cvetanja. Ovde pokazujemo da i neindukcioni fotoperiod koji prethodi indukciji cvetanja materinskih biljaka pokazuje materinski efekat na potomstvo: veličinu semena, klijanje semena i sastav proteina semena. Prikazani rezultati govore u prilog ranije predloženog: relativna količina proteina semena predstavlja "arhivu" fotoperioda koju je majka biljka iskusila tokom svog životnog ciklusa.

Key words: *Chenopodium rubrum*, materinski efekat fotoperioda, proteini semena, masa semena, klijanje semena

Introduction

Annual plants experience a single environment during their life cycle, but they could be exposed to different environmental conditions through environmental changes. Environmental effects on morphological and physiological properties of offspring which occurs during development of mother plant are called maternal environmental effects (Gutterman & Evenari, 1972; Galloway, 2005). Their expression depends on the offspring environment, they are expressed throughout the life cycle of the offspring and may persist for several generations. Maternal environmental effects could be provoked by different environmental factors such as soil nutrients (Straton, 1989), temperature (Lacey et al., 1997), photoperiod (Cook, 1975; Gutterman, 1978; Berttero et al., 1999; Mitrović et al., 2010), CO_2 levels (Steinger et al., 2000).

The day length (night length), i.e. photoperiod, is one of the most crucial environmental factors according to which plants modify numerous physiological processes, the transition to flowering being one of the most important turning points in mother plant life cycle in order to provide the success of its offspring. In species with strong maternal effects, maternal plants could be grown under appropriate environmental conditions to promote successful establishment of new populations. For example, in the case of assisted dispersal to habitats with longer day length, maternal plants can be grown under the appropriate day length so maternal effects are adaptive (Schuler & Orrock, 2012). On the other hand, if the seeds disperse into a different habitat, maternal environmental effects may reduce the offspring fitness, but only for a single generation, since the response to the new habitat will induce new maternal effects (Galloway, 2005).

Chenopodium rubrum L. sel. 184 is a qualitative short day (SD) weedy annual (Cumming, 1967) with well defined photoperiodic sensitivity. It is sensitive to the small changes in day length, with defined critical night length of 8 h (Tsuchia & Ishiguri, 1981). *C. rubrum* is sensitive to photoperiodic stimulus for flowering as early as at cotyledonary stage (Seidlová & Opatrná, 1978), when 6 adequate photoperiodic cycles are sufficient for photoperiodic flower induction. It is an early flowering species (Cumming, 1967) which makes it a suitable model plant for studies of ontogenesis. Under the suitable photoperiodic conditions *in vitro* it produces seeds in 10 weeks (Mitrović et al., 2007). *C. rubrum* plants modify their vegetative and reproductive development, in accordance with the photoperiod they are exposed to (Cook, 1975; Mitrović et al., 2007). Its response to photoperiod extends to the offspring and persists to the second generation, the suggested mechanism of maternal effect of photoperiod being through seed protein composition (Mitrović et al., 2010).

In *C. rubrum*, as a SD plant able to receive photoperiodic flowering induction as early as at about 5 days of age, most of the data concerning maternal effect of photoperiod on offspring deal with photoperiods inductive for flowering and its alterations on the plants of the same age and early during their life (Cook, 1975; Mitrović et al., 2010). Hence, maternal effect of different photoperiods inductive for flowering on different phases of offspring ontogenesis (Mitrović et al., 2010) is defined. *C. rubrum* seed number and seed weight is determined by photoperiod mother plants are exposed to during induction and evocation of flowering (Cook, 1975; Mitrović et al., 2007). Seed germination and offspring growth is determined by photoperiod during flowering induction of mother plants, offspring flowering and seed maturation is determined by photoperiod their mothers experienced during induction and evocation of flowering, while photoperiod after the evocation of flowering of mother plants does not affect offspring ontogenesis significantly (Mitrović et al., 2010). On the other hand, in nature, *C. rubrum* plants flowers when summer days become shorter. This means that they receive photoperiodic induction for flowering later during their life and thus under the longest photoperiods physiologically possible (Cook, 1975). In other words, in nature, transition from vegetative to reproductive development (flowering induction) occurs when night length becomes longer than its critical night length of 8 h (Tsuchia & Ishiguri, 1981). In this regard, the information about timing in offspring life when photoperiod inductive for flowering is to be expected could be of great importance for offspring success. Therefore, it is to be expected that besides photoperiod during induction and evocation of flowering of mother plants, also non-inductive photoperiod preceding flowering induction of mother plants and its duration shows its (maternal) effect on offspring. Cumming (1967) observed significant decrease in seed size if SD plants from genus *Chenopodium* were grown under non-inductive photoperiod preceding flowering induction compared to those grown continuously under inductive short days. But there is no more precise data of the effect of non-inductive photoperiod preceding flowering induction on offspring properties.

Here we present preliminary data on maternal effect of non-inductive photoperiod preceding flowering induction on seed properties: seed size, seed germination and variation in seed protein pattern. *In vitro* culture of intact plants was selected because it enables precise control over environmental factors, as it was shown that other environmental factors, such as temperature, significantly affects maternal effect of photoperiod on different *Chenopodium* species (Bertero et al., 1999; Mitrović et al., 2007). As non-inductive photoperiod for obligatory SD plant *C. rubrum*, continuous light (CL) was selected as extreme long day (Cumming, 1967; Mitrović et al., 2007). The duration of non-inductive photoperiod preceding flowering induction was chosen to be longer than the period necessary for *C. rubrum* plants to reach full flowering under inductive 14 h/10 h photoperiod *in vitro* (Živanović et al., 1995). Finally for photoperiod inductive for flowering 14 h/10 h was used, established as inductive in *C. rubrum* flowering research (Krekule & Seidlova, 1976; Seidlová & Opatrná, 1978; Živanović et al., 1995).

Material and methods

Plants *in vitro*

Intact *C. rubrum* plants were grown *in vitro* on MS (Murashige & Skoog, 1962) medium, as described in Mitrović et al. (2007). Seedlings, 5 days old, were exposed to two different photoperiodic treatments: 65 days of a 14 h/10 h photoperiod or 17 days of continuous light followed by 43 days of 14 h/10 h (Mitrović et al., 2008). Irradiance was about 70 µmol m^{-2} s^{-1}. Temperature in the growth chambers was 25 ± 2 ºC.

Seed collection

Matured seed were collected, dried for 1 month at room temperature, measured (4 replicates of 100 seeds) and capped on +8°C until use 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.

Seed extraction

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 ml 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 concentration

Protein concentration in seed samples derived from mother plants grown under different photoperiodic conditions was determined by Bradford (1976) method with bovine serum albumin as the standard.

Separation of seed proteins

Protein separation, in seed samples derived from mother plants grown under different photoperiodic conditions, was performed on sodium dodecilsulfate poliacrilamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). Polyacrylamide gel electrophoresis was carried out under non/denaturing conditions in gels containing 10% polyacrylamide with a 4% stacking gel. A constant current of 25 (15) mA per gel was applied. Equal volumes of all samples were loaded into the gels. Proteins were visualized by Coomassie blue staining. For SDS-PAGE separation all samples were run in triplicate. Relative values of seed protein band intensities were determined in Image Master Totallab 1.11.

Seed germination

Seeds were sown on moistened filter paper (5 ml distilled water) in Petri dishes. Germination was tested during 4 days (24 h dark at 32 °C, 24h dark at 10 °C and 48 h white light at 32 °C). Every 24 h, 4 replicates of 100 seeds per treatment of mother plant were scored for germination. As a criterion of germination, radical protrusion by more than 2 mm was used.

Results and discussion

For an obligatory SD plant, such as *C. rubrum*, extreme long day (CL) is non-inductive photoperiod, under which it grows continuously vegetative (Cumming, 1967; Mitrović et al., 2007).

Analyzing literature concerning the effect of CL on plant growth and development Sysoeva et al. (2010) showed that there are reports of CL both increasing plant developmental rate and inhibiting it. Plant developmental response to CL depends on many factors including photoperiodic sensitivity, developmental stage and environmental conditions. In most long-day plants CL accelerated the reproductive cycle, while SDPs responded to CL differently. On the other hand growing plants under CL is a way of economical crop production. It also provides better understanding of plant adaptations to the Arctic polar 24 h photoperiod (Sysoeva et al., 2010).

Maternal effect of non-inductive extreme long days, CL, preceding *C. rubrum* flowering induction on seed weight

In previous work (Mitrović & Bogdanović, 2008), we showed that about 5 times more seeds were collected from plants in which 17 non-inductive extreme long days (CL) preceded flowering induction by 14 h /10 h photoperiod, compared to those grown continuously under inductive 14 h /10 h photoperiod (Mitrović & Bogdanović, 2008). As opposed, seed weight is for one third lower if collected from mother plants in which CL preceded flowering induction (**Fig. 1A**).

Hence, we show significant difference in weight of seeds collected from mother plants induced for flowering under the same 14 h /10 h photoperiod, but with the time span of 17 days in their life cycle. This confirms Cumming (1967) observation that SD plants from the genus *Chenopodium*, grown under long days or CL before flowering induction, produces smaller seeds compared to seeds collected from plants grown continuously under the short days. On the other hand, on the basis of the experiments in which *C. rubrum* mother plants were grown continuously under different inductive photoperiods, it was shown that seed number and seed weight is determined by photoperiod mother plants experienced during induction and evocation of flowering (Cook, 1975; Mitrović et al., 2007).

From the abovementioned arises that seed size and weight is determined not only by the photoperiod under which induction and evocation of

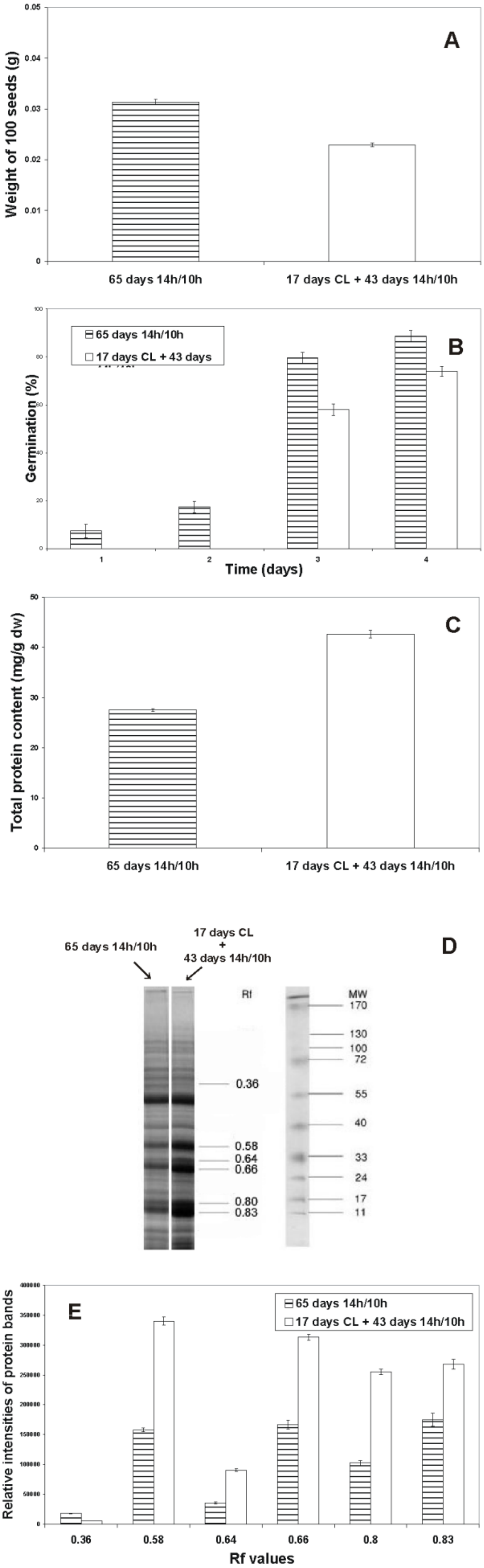

Fig. 1. Maternal effect of 17 days of continuous light (CL), extreme non-inductive photoperiod, preceding flowering induction on offspring properties in a qualitative short day plant *Chenopodium rubrum* on: A) seed weight, B) seed germination, C) total seed protein content, D) width and intensity of seed protein bands (coomassie blue stained SDS-PAGE gel, Rf values are marked for seed protein bands for which the difference in width and intensity are noticed between seed samples collected from mother plants grown continuously under inductive 14 h /10 h photoperiod and those in which 17 days of non-inductive CL preceded flowering induction by 14 h /10 h photoperiod), and E) relative intensities of seed protein bands (determined by Image Master Totallab 1.11)

flowering of mother plants occurred (Cook 1975; Mitrović et al., 2007), it is also significantly affected by non-inductive photoperiod preceding flowering induction of mother plants.

Maternal effect of non-inductive extreme long days, CL, preceding *C. rubrum* flowering induction on seed germination

Fig. 1B shows that CL preceding flowering induction of mother plants also affect seed germination. Germination of small seeds collected from mother plants in which 17 days of CL preceded flowering induction, is delayed and synchronized compared to germination of seeds collected from mother plants grown continuously under inductive 14 h/10 h photoperiod (**Fig. 1B**). Also, Cumming (1967) showed that in small seeds, obtained from mother plants grown under non-inductive long days or CL before flowering induction were more dormant, the delay in germination happens due to thicker seed integument. At the same time, germination of seeds collected from mother plants grown continuously under different inductive photoperiods was determined by photoperiod experienced during flowering induction of mother plants (Mitrović et al., 2010).

So, the same as seed size and weight, seed germination is, as well, determined not only by the photoperiod under which induction and evocation of flowering of mother plants occurred, but also is significantly affected by photoperiod preceding flowering induction of mother plants.

Maternal effect of non-inductive extreme long days, CL, preceding *C. rubrum* flowering induction, on seed proteins

Although seed storage proteins in *Chenopodium* species are localized mostly in endosperm and embryo (Prego et al., 1998), while the difference in seed size are due to proportional difference in both the size of the embryo and endosperm in (Cook, 1975), protein content (**Fig. 1C**) was nearly twice higher in smaller seeds (**Fig. 1A**) collected from mother plants in witch flowering induction was preceded by 17 days of CL.

We suggested earlier that the mechanism of maternal effect of photoperiod can be through relative seed protein composition representing an “archive“ of photoperiods experienced by mother plants during their lives (Mitrović et al., 2010). Bhargava et al. (2005) showed the difference in number, width and intensity of seed protein bands on SDS-PAGE gel, not only in seeds samples of different *Chenopodium* species, but also in seed samples of the same species collected from widely separated localities (characterized by highly variable environmental factors). **Fig. 1D** shows the presence of 33 protein bands on SDS-PAGE gel in both *C. rubrum* seed samples. The same number of protein bands was previously obtained (Mitrović et al., 2010) in seed samples collected from mother plants grown continuously under different inductive photoperiods *in vitro*. This confirmes that the difference in quantities of those 33 seed protein bands is only the result of different photoperiods mother plants were exposed to during their life cycle, as grown under precisely controlled environment *in vitro*. For 6 of them (**Fig. 1E**), significant differences in width and intensity were noticed. Protein bands with Rf values 0.58, 0.64, 0.66, 0.80 and 0.83 showed about twice higher relative intensities in small seeds collected from mother plants in which flowering induction was preceded by 17 days of CL (**Fig. 1E**). Hence, in seeds collected from mother plants in witch flowering induction was preceded by CL, higher protein content (**Fig. 1C**) is a result of increased amounts of those protein bands (with Rf values 0.58, 0.64, 0.66, 0.80 and 0.83). At the same time, for those protein bands we showed earlier high correlations with the day length mother plants experienced during induction and evocation of flowering (Mitrović et al., 2010). Therefore, this is the additional confirmation that relative amounts of those specific seed proteins are dependent on photoperiods experienced by mother plants during their lives.

Conclusion

C. rubrum is a species with strong maternal effects. Besides photoperiod during induction and evocation of flowering of mother plants (Mitrović et al., 2010), here we show that also non-inductive photoperiod preceding flowering induction of mother plants shows its maternal effect on offspring properties. Maternal effect of non-inductive, extreme long days, CL, preceding flowering induction of *C. rubrum* mother plants results in lowering seed size, enhancing seed dormancy, and increase in the amounts of some specific seed proteins. This goes in favor of earlier suggested that seed proteins represent an “archive“ of photoperiods experienced by mother plants during their lives. Detailed analysis of altering non-inductive photoperiods and its duration preceding *C. rubrum* flowering induction, as well as identification of seed proteins we found dependant on photoperiods experienced by mother plants, would contribute understanding the mechanism of maternal environmental effects.

Acknowledgements. This work was financed by the grants ON173017 and III43010 from the Ministry of the Education and Science of the Republic of Serbia.

References

Bertero, H.D., King, R.W., Hall, A.J. 1999: Photoperiod-sensitive development phases in quinoa (*Chenopodium quinoa* Willd.). *Field Crops Research*, 60: 231-243.

Bradford, M.M. 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye binding. *Analitical Biochemistry*, 72: 248-254.

Cook, R.E. 1975: The photoinductive control of seed weight in *Chenopodium rubrum* L. *American Journal of Botany*, 62: 427-431.

Cumming, B.G. 1967. Early flowering plants. In: F.H. Will and N.K. Wesselss, Thomas Y. Cromwell, (eds.), Methods in developmental biology: 277-299, New York.

Galloway, L.F. 2005: Maternal effects provide phenotipic adaptation to local environmental conditions. *New Phytologist*, 166: 93-100.

Gutterman, Y., Evenari, M. 1972: The influence of day length on seed coat colour, an index of water permeability of the desert annual *Ononis sicula* Guss. *The Journal of Ecology*, 60: 713–719.

Gutterman, Y. 1978: Germinability of seeds as a function of the maternal environment. *Acta Horticulturae*, 83: 49-56.

Krekule, J., Seidlova, J. 1976: Effects of exogenous cytokinins on flowering of the short-day plant *Chenopodium rubrum* L. *Biologia Plantarum*, 18: 142-149.

Lacey, E.P., Smith, S., Case, A.L. 1997: Parental effects on seed mass: seed coat but not embryo/endosperm effects. *American Journal of Botany*, 84: 1617-1620.

Mitrović A, Giba Z., Ćulafić Lj. 2007: The photoperiodic control of growth and development of *Chenopodium rubrum* L. plants *in vitro*. *Archives of Biological Sciences*, 59: 203-208.

Mitrović, A., Bogdanović, J. 2008: Activities of antioxidative enzymes during *Chenopodium rubrum* L. ontogenesis *in vitro*. *Archives of Biological Sciences*, 60: 223-231.

Mitrović, A., Bogdanović, J., Giba, Z., Ćulafić, Lj. 2010: Effect of photoperiod during growth of *Chenopodium rubrum* mother plants on properties of offspring. *Biologia Plantarum*, 54: 735-739.

Murashige, T., Skoog, F. 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473-497.

Prego, I., Maldonado, S., Otegui, M. 1998: Seed structure and localization of reserves in *Chenopodium quinoa*. *Annals of Botany*, 82: 481-488.

Schuler, M.S, Orrock, J.L. 2012: The maladaptive significance of maternal effects for plants in anthropogenically modified environments. *Evolutionary Ecology*, 26:475–481.

Seidlová, F., Opatrná, J. 1978: Change of growth correlation in the shoot meristem as the cause of dependance of flowering. *Zeitschrift fur Planzenphysiologie*, 89: 377-392.

Steinger, T.B. 2000: Maternal and direct effects of elevated CO_2 on seed provisioning, germination and seedling growth in *Bromus erectus*. *Oecologia*, 123: 475-480.

Straton, D.A. 1989: Competition prolongs expression of matenal effects in seedlings of *Erigeron annuus* (Asteraceae). *American Journal of Botany*, 76: 1646-1653.

Sysoeva, M., Markovskaya, E. F., Shibaeva, T.G. 2010: Plants under continuous light: A review. *Plant Stress*, 4: 5-17.

Tsuchiya, T., Ishiguri, Y. 1981: Role of the quality of light in the photoperiodic flowering response in four latitudinal ecotypes of *Chenopodium rubrum* L. *Plant and Cell Physiology*, 22: 525-532.

Živanović, B., Ćulafić, Lj., Filipović, A. 1995: The effects of hormones and saccharides on growth and flowering of green and herbicides-treated *Chenopodium rubrum* L. plants. *Biologia Plantarum*, 37: 257-264.