BIOLOGICA NYSSANA
1 (1-2) • December 2010: 61-64 10th SFSES • 17-20 June 2010, Vlasina lake

Original Article

Chenopodium rubrum L. as a model plant for physiological and biochemical investigations of ontogenesis *in vitro*

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***Abstract*:**
Mitrović, A., Živanović, B., Dučić, T., Bogdanović-Pristov, J., Radotić Hadži-Manić, K.: *Chenopodium rubrum L. as a model plant for physiological and biochemical investigations of ontogenesis in vitro. Biologica Nyssana, 1 (1-2), December 2010: 61-64.*

Chenopodium rubrum L. , is a suitable model plant for studying ontogenesis *in vitro* as an early flowering species. Culture of intact plants *in vitro* and antioxidative enzymes detection were performed. Growth pattern to the end of ontogenesis, flowering and seed development are all determined by the photoperiod seedlings experience during induction and evocation of flowering. Different phases of vegetative and reproductive development are characterized by changes in antioxidative enzymes activities. We showed sequential expression of antioxidative enzymes during seed germination. Prior to radicule protrusion, CAT and SOD showed maximal activity, while POD activity appeared later. The highest catalase (CAT) activity was measured at the time of flowering while peroxidases (PODs) are involved in determination of growth and development in accordance with the environmental clues. The absence of some superoxide dismutase (SOD) isoforms could be the indicator of senescence. Seed ageing affect changes in antioxidative status of seeds, germination, seedling growth and flowering.

Key words: catalase, *Chenopodium rubrum*, germination, flowering, peroxidase, superoxide dismutase

Introduction

Chenopodium rubrum L. belongs to the family Chenopodiaceae, genus *Chenopodium*. This is a short-day weedy annual, widely distributed in Europe, Asia and Northern America. Ecotypes of this species differ in their photoperiodic characteristics. Sel. 184 is a qualitative short-day plant with strictly defined critical night length of 8h (Tsuchiya & Ishiguri, 1981). It 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. *C. rubrum* plants modify their growth and development in accordance with photoperiod they are exposed to (Cook, 1975; Mitrović et al., 2007). As an early flowering species (Cumming, 1967), it is a suitable model plant for studying ontogenesis *in vitro*. Under the adequate photoperiodic conditions, plant flowers *in vitro* after 15 days (Živanović et al., 1995), and produces seeds after 10 weeks (Mitrović et al., 2007). Moreover different phases of vegetative and reproductive development are characterized by changes in antioxidative enzymes activities. Thus seed germination, seedling growth, flowering, seed maturation and seed ageing are defined by the changes in their antioxidative status (Mitrović, 2007).

It is well known that reactive oxygen species (ROS) and their scavenging enzymes participate in protection against pathogens or abiotic stress (Hendry & Crawford, 1994). ROS also function as signaling molecules at low

concentrations in contrast to high concentrations of ROS which can lead to phytotoxicity (Foyer, 1997). The capacity of ROS to serve as signaling molecules highlights the importance of antioxidants to specifically regulate different ROS in various cellular compartments. The lack of data obtained from ROS measuring in plants is due to technical difficulties associated with quantification of endogenous levels of these very reactive and short-lived species. So, most of the evidences for ROS levels has ben provided by studies of antioxidants (Dat et al., 2000). Antioxidative enzymes, catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) are engaged in the scavenging of ROS (Van Loon, 1986; Bowler et al., 1992; Khan & Panda, 2002) and therefore participate in regulation of plant growth and developmental processes or protection against patogens or abiotic stress. SOD plays a crucial role in the antioxidative system by catalysing dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2. CAT has a high reaction rate, but a low affinity for H_2O_2, thereby removes the bulk of H_2O_2. Inversely POD has a higher affinity for H_2O_2, allowing for the scavenging of small amounts of H_2O_2 in more specific locations (Dat et al., 2000). Changes in CAT activity are linked to desiccation during seed maturation (Bailly et al., 2004), seed germination (Bailly et al., 2002; Prodanović et al., 2007; Bogdanović et al., 2008) and plant growth and development (Bailey & Mc Hargue, 1943; Matters & Scandalios, 1986; Mitrović & Bogdanović, 2008). PODs are the most investigated enzymes since they have a role in very important physiological processes like seed germination, seedling growth (Belani et al., 2002; Dučić et al., 2003/4; Prodanović et al., 2007; Bogdanović et al., 2008), root growth (Kukavica et al., 2007), plant growth and development (Bailey & Mc Hargue, 1943; Mitrović & Bogdanović, 2008), and lignin biosynthesis in cell walls (Bruce & West, 1989).

This specific review summarizes some of the data obtained on model plant *Chenopodium rubrum* L. in our laboratory in order to improve the understanding of involvement of antioxidative enzymes activities (and circumstantially ROS) in regulation of *C. rubrum* vegetative and reproductive development.

Results and disscusion

Seed germination

Seed germination starts with imbibition, and ends with radicule protrusion. It is a complex process, associated with many metabolic, cellular and molecular events. Accumulation of reactive oxygen species (ROS), during seed imbibition, leads to germination (Bailly et al., 2004). Therefore, antioxidant enzymes have a particular importance for the completion of germination. Protein content increased during *C. rubrum* germination (Dučić et al., 2003/4), since proteins are both released from protein storage or synthesized *de novo* during and after the imbibition phase of germination, as building and regulatory material in emerging seedlings (Roberts, 1972). During *C. rubrum* seed germination sequential expression of antioxidative enzymes occurred (Dučić et al., 2003/4). CAT and SOD showed the highest activity at the time preceding radicule protrusion, while significant expression of POD occurred after this term. Increase in POD activity corresponds to the expression of new POD isoforms (Dučić et al., 2003/4). The appearance and increase of POD activity during germination could be specifically linked with final phases of seed germination or early seedling growth (Schopfer et al., 2001; Dučić et al., 2003/4; Bogdanović et al., 2008).

Sequential expression of antioxidative enzymes during *C.rubrum* seed germination points out that the decrease in H_2O_2 level coincide with final phases of seed germination and early seedling growth.

The photoperiodic control of growth and development

As already stated, in *C. rubrum* sel. 184 critical night length and sensitivity to photoperiod are well defined (Tsuchiya & Ishiguri, 1981; Seidlová & Opatrná, 1978). Altering day length in such a plant, is a valuable source of information about regulation of plant development in accordance with the photoperiod. Thus with the increase of day length, plant height is increased, flowering is delayed, seed development occured earlier, and plants produced more seeds (Mitrović et al., 2007). *C. rubrum* growth pattern to end of ontogenesis, flowering and seed development, are all determined by the photoperiod the seedlings experience during early phases of reproductive development - induction and evocation of flowering (Mitrović et al., 2007; Cook, 1975). Natural *C. rubrum* flowering induction works in line with minimizing seed weight and maximizing seed number, favorizing physiological mechanisms that works under suboptimal photoperiods, maximizing probability to survive (Cook, 1975). In addition to photoperiod temperature also affected seed weight, as previously reported for *Chenopodium quinoa* (Bertero et al., 1999) and *C. rubrum* (Mitrović et al., 2007).

Antioxidative enzymes activities during ontogenesis *in vitro*

By altering photoperiods in plants with well defined critical night length and sensitivity to photoperiod, it is possible to separate on the time scale different developmental phases (vegetative growth, flowering, seed development and maturation) in plants of the same age.

The activities of antioxidative enzymes changes with both, phase of development and photoperiod plants are exposed to (Lall & Nikolova, 2003; Mitrović & Bogdanović, 2008). *C. rubrum* flowering *in vitro* was associated with the highest CAT activity. The intensities of 17 POD isoforms differed with day length plants were exposed to. So, it was suggested that PODs are involved in determination of *C. rubrum* growth and development in accordance with seasonal changes of day length. POD isoform pI 4.6 could be associated with stress, induced both, by exposure to continuous light and by senescence *(*Mitrović & Bogdanović, 2008). According to previously reported data for *Arabidopsis thaliana* it was shown that $^{\cdot}O_2^-$ is involved in the induction and development of senescence (Abarca et al., 2001). The absence of some SOD isoforms could be the indicator of *C. rubrum* senescence *in vitro* which starts during the phase of seed maturation (Mitrović & Bogdanović, 2008).

Seed aging

Seed aging is a natural process, starting during, or shortly after harvesting due to irreversible changes leading to loss of viability (Villiers, 1972). During seed aging chromosome and membrane damage occurs, as well as the damage of the enzyme structure, caused by ROS. In the same time ROS have the functional significance in seed ageing and germination (Schopfer et al., 2001). Seeds sampled during ageing showed an increase in the time needed to start germination possibly due to the need for repair and replacement processes to occur. Protein *de novo* synthesis starts later during imbibition in aged seeds (Villiers, 1972). Protein content, as well as CAT and SOD activity, was lower in aged *C. rubrum* seeds (2.5h darkness imbibed), compared to young seeds (Mitrović et al., 2005). Viability declines with seed aging (Bewley & Black, 1982) and germination of 3 years old *C. rubrum* seeds was delayed compared to 3 months old ones (Mitrović et al., 2005). So, antioxidant enzyme activities may be involved in the evaluation of seed viability in seed ageing. Seed age also affect seedling growth and flowering. Plants derived from aged seeds shows inhibited growth *in vitro* (Mitrović et al., 2005), and delayed flowering (Mitrović et al., 2005; Kadman-Zahavi & Peiper, 1987).

Conclusion

This review has summarized some of data obtained on model plant *Chenopodium rubrum* L. grown *in vitro* in our laboratory during last decade. Our results improved knowledge of *C. rubrum* sensitivity to day length and also confirmed the involvement of antioxidative enzymes in regulation of plant development, from seed germination to seed maturation.

Acknowledgments. This work was supported by a grant (№. 143043) from the Ministry of Science and Technological Development, Republic of Serbia.

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