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Original Article

Chenopodium murale L., a long-day plant as a model for physiological and biochemical research

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***Abstract*:**
Živanović, B., Mitrović, A., Bogdanović-Pristov, J., Radotić Hadži-Manić, K., Ćulafić, Lj.: *Chenopodium murale L., a long-day plant as a model for physiological and biochemical research. Biologica Nyssana, 1 (1-2), December 2010: 71-75.*

Chenopodium murale L. genus *Chenopodium* family Chenopodiaceae is a weedy annual widely distributed in Serbia. This is a ***long-day plant and*** an early flowering species. We used culture of intact plants *in vitro* and antioxidative enzymes detection in order to examine the effect of gibberellic acid (GA_3) on two key processes during ontogenesis – germination and flowering. Our results showed a sequential expression of antioxidative enzymes during germination. In dry seeds and during early imbibition catalase (CAT) and superoxide dismutase (SOD) activities showed no changes, while peroxidase (POD) activity was under the level of delectability. During radicule protrusion CAT and SOD activity increased. Early seedling development correlates with decrease in SOD, increase in CAT and appearance of POD activity. GA_3 delayed and synchronized germination. *C. murale* photoperiodic sensitivity for flowering shows age-dependant oscillatory changes. Glucose and GA_3 have cumulative stimulatory effect on its flowering *in vitro*. The exposure of 2 months old vegetative plants to continuous darkness, in the presence of GA_3 in culture media, resulted in flowering. Therefore transferring to darkness canceled photoperiodic control in *C.murale* and flowering occurred under autonomous mechanism. We suggest *C.murale* as a suitable model for investigation of physiological and biochemical mechanisms of growth and developmental processes.

Key words: catalase, *Chenopodium murale* L., germination, gibberelic acid, flowering, peroxidase, superoxide dismutase

Introduction

Chenopodium murale L. belongs to the family Chenopodiaceae, genus *Chenopodium*. Ecotype 197 is characterized by Cumming (1967) as a facultative long-day annual and an early flowering species. It is sensitive to photoperiodic flowering induction as early as at the phase of the 1st pair of leaves (Pavlová et al., 1989). 10 cycles of continuous light (24 h light) are necessary for photoperiodic flowering induction, regardless of plant age (Pavlová et al., 1989). Intact *C. murale* plants, cultured *in vitro* on optimal media composition and under adequate photoperiodic regime, produces seeds in 18 weeks.

Gibberellins (GAs) are very potent plant growth regulators. GAs plays an important role in stimulation of seed germination (Bewley, 1997). They are efficient in breaking seed dormancy, by their ability to overcome the requirements for environmental factors, and in accelerating germination in non-dormant seeds (Bewley &

Black, 1982). GA_3 also induces or promotes flowering, being particularly effective in long-day species (Evans et al., 1999; Živanović et al., 1995; Mitrović et al., 2000; Mitrović et al., 2000; Mitrović et al., 2003).

Continuous production and removal of reactive oxygen species (ROS) is, besides as a phenomenon with negative consequences (damage of cell membranes and organelles), linked with a signaling role in plant developmental processes. Antioxidative enzymes, catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) are engaged in the scavenging of ROS (Dat et al., 2000) and therefore participate in regulation of plant growth and development (Mitrović, 2007) as well as in the protection against pathogens or abiotic stress (Hendry & Crawford, 1994).

This is a review of our previously reported data that describe the effects of gibberellic acid in regulation of *C. murale* seed germination and flowering, as two key processes in plant ontogenesis.

Results and disscusion

Seed germination

Seed germination starts with imbibition, and ends with radicule protrusion. It can be divided in three phases: imbibition (rapid initial water uptake – physical process characteristic also for dead seeds), the plato phase (small change of water content but high metabolic activity) and further water uptake coinciding with radicule protrusion and growth (Giba et al., 2004). Plato phase, as the most important in regulation of germination, involves the activation of specific enzymes at the appropriate time and regulation of their activity (Riley, 1987). It was supposed that accumulation of reactive oxygen species (ROS), during seed imbibition, leads to germination (Bailly, 2004). Therefore, antioxidant enzymes have been considered to be of particular importance for the completion of germination.

Sequential expression of antioxidative enzymes and their importance during germination in different plant species was shown (Dučić et al., 2003/4; Bailly et al., 2000; Puntarulo et al., 1991; Bogdanović et al., 2008). Gibberellic acid delayed and synchronized *C. murale* germination and showed similar effect on protein content and activities and isoenzyme pattern of antioxidative enzymes (Fig. 1, Fig. 2) (Bogdanović et al., 2008). This suggests that GA_3 and ROS, thorough activities of antioxidative enzymes, participate in the same signaling pathway in germination.

Protein concentration increased at the time of *C.murale* ridicule protrusion (Bogdanović et al., 2008), connected with release from storage and *de novo* synthesis of building and regulatory proteins in the emerging seedlings (Roberts, 1972).

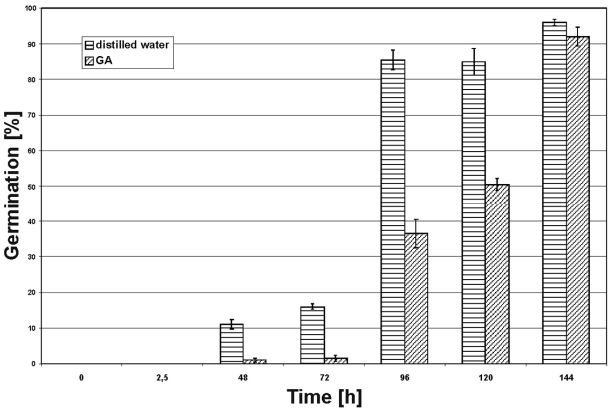

Fig. 1. *C. murale* germination (%) on distilled water or GA_3 (160 μM) solution. Data points are averages of four replicates of 50 seeds each. Modified from Bogdanović et al., 2008

In dry seeds and during early imbibition catalase (CAT) and superoxide dismutase (SOD) activities showed no changes, while peroxidase (POD) activity was under the level of delectability (Fig. 2). CAT activity increased during radicule protrusion and early seedling development, by appearance of a new CAT isoform. Pick in SOD activity coincided with radicule protrusion and early seedling development. Based on the specific inhibition of SOD activity by KCN and H_2O_2, we showed that *C.murale* seeds/seedlings contain only Mn SOD form. POD activity appeared prior to or simultaneously with radicule protrusion and increased during early seedling growth, presented with two basic isoforms (Bogdanović et al., 2008). Specific role of PODs in regulation of final phases of germination and early seedling growth was suggested in many species (Schopfer et al., 2001; Dučić et al., 2003/4; Prodanović et al., 2007; Bogdanović et al., 2008). PODs have a very important role in physiological processes in plants (Gaspar et al. 1991), but their exact relationship to developmental events is often obscure by their extensive polymorphism in a single plants species.

Changes in CAT, SOD and POD activities could be "the markers" of different phases of germination. Decrease in SOD activity (H_2O_2 producing enzyme), increase in CAT and appearance of POD activity (H_2O_2 consuming enzymes) coincide with early seedling development in *C. murale.* These data, accompanied with similar findings concerning seed germination in different plant species, suggest that decrease in H_2O_2 level

might be involved in regulation of this process (Bogdanović et al., 2008).

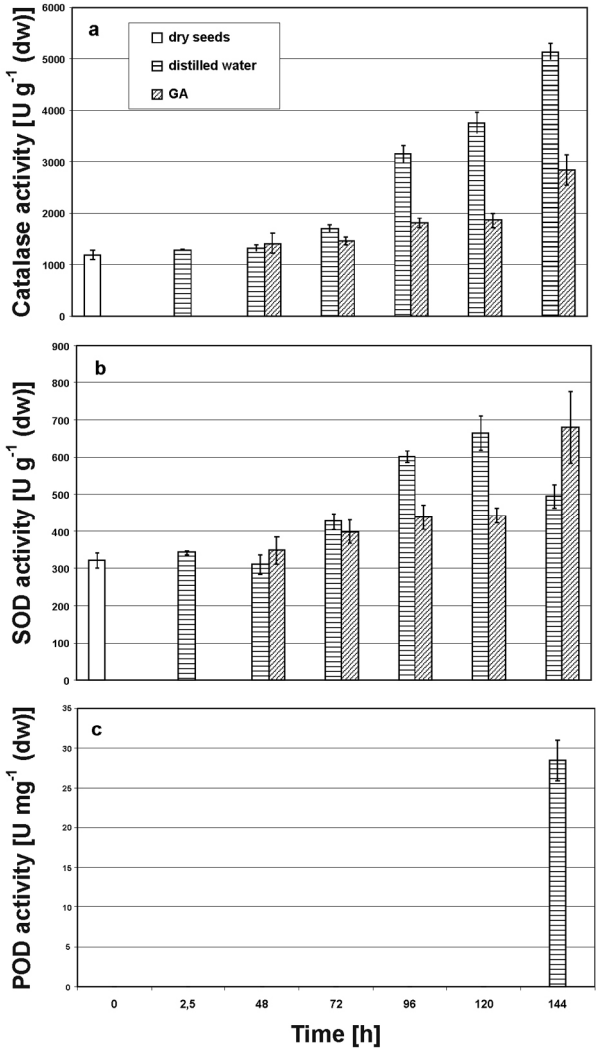

Fig. 2. Catalase, superoxide-dismutase and peroxidase activities [mg g^{-1} (d.w.)] during *C. murale* seed germination on distilled water or GA_3 (160 μM) solution. Modified from Bogdanović et al., 2008

Flowering

Transition from vegetative to reproductive phase of development is controlled by genetical (autonomous) and ecological (photoperiod and/or temperature) factors. Autonomous control that depends on plant age is the basic control level. Induced or photoperiodic control, alone or in combination with temperature, is the second level of flowering control that stimulates or inhibits genetically determined flowering.

In *C.murale* plants photoperiodic sensitivity is age-dependant. It shows oscillatory changes with aging (expressed by number of leaves) (Pavlová et al., 1989; Mitrović et al., 2000). 10 cycles of continuous light (24 h light) are necessary for photoperiodic flower induction, regardless of plant age (Pavlová et al., 1989; Mitrović et al., 2000). Glucose and GA_3 have cumulative stimulatory effect on *C.murale* flowering *in vitro* under inductive photoperiodic conditions, probably affecting flower development, rather than flower initiation (**Tab. 1**). Under photoperiod non-inductive for flowering neither glucose nor GA_3 were able to compensate for *C.murale* photoperiodic requirements for flowering (Mitrović et al., 2000).

The photoperiodic control is loosened with aging in some plants (*Perilla nankensis, Rudbeckia bicolor*) (Bernier et al., 1981; Chailakhyan, 1988). Exposure to darkness cancels photoperiodic control, so the flowering is controlled only by the autonomous mechanism (Chailakhyan, 1988; Mitrović et al., 2003). Some long-day and short-day plants produces flowers under continuous darkness (Fife and Price, 1953; Sugino, 1957; Inouye et al., 1964; Takimoto, 1960; Mitrović et al., 2003; Cvetić et al., 2004). In 5 – 7 month old long-day plant *Rudbeckia bicolor* flowering occurred after exposure to darkness (Chailakhyan, 1988). In 2 months old *C. murale* plants, exposed to continuous darkness for 10 days, the addition of GA_3 in culture medium was necessary for transition to flowering (**Tab. 1**) (Mitrović et al., 2003). GA_3 alone was not able to compensate for *C. murale* photoperiodic demands for flowering. So we suggest that GA_3 affected flower development, rather than flower initiation. *C.murale* plants partly loose demands for specific day length with aging, so transferring to darkness cancels photoperiodic control and flowering occurs under autonomous mechanism (Mitrović et al., 2003).

Conclusion

This is a review of our work on model plant *Chenopodium murale.* Our results improved the knowledge of the role of gibberellic acid in two key processes during ontogenesis - germination and flowering. GA_3 delay and synchronize germination, while it stimulates flowering. Changes in activities and isoforms of antioxidative enzymes could be "the markers" of different phases of seed germination. Intact *C.murale* plants have the ability to be induced for flowering in total darkness *in vitro*.

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Table 1. Flowering of *C. murale* plants *in vitro.* Plants were induced for flowering at different age (expressed by number of leaves) by exposure to 10 days of continuous light or 10 days of continuous darkness; dCL – days of continuous light (24 h light), dCD – days of continuous darkness (24 h darkness), SD – short day (8/16 h light/darkness). Modified from Mitrović et al., 2000 and Mitrović et al., 2003.

Photoperiodic conditions	GA_3 (mg dm^3)	Flowering (%)
10 dCL in the phase of development of 1st pair of leaves 12 SD + 10d CL +27 SD	0	17
	1	40
	5	43
10 dCL in the phase of development of 2nd pair of leaves 29 SD +10 dCL + 30 SD	0	0
	5	13
10 dCL in the phase of development of 3rd pair of leaves 42 SD + 10 dCL + 30 SD	0	18
	5	67
10 dCL in the phase of development of 4th pair of leaves 60 SD +10 dCL + 30 SD	1	60
10 dCD in the phase of development of 4th pair of leaves 60 SD + 10 d CD + 30 SD	0	0
	1	42
	5	65

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