Peroxidase isoenzymic spectra of cabbage, kohlrabi and red beet during flowering induction and generative development

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The dynamics of peroxidase isoenzymes composition in various organs of cabbage 'Early Golden Acre', kohlrabi 'White Vienna' and red beet 'Kamuoliai 2' during ontogenesis was investigated. The largest number of isoforms was detected from the juvenile stage till the end of evocation in cabbage (5 isoforms), at the end of evocation and during flower initiation in kohlrabi (7 isoforms), from the beginning of flowering induction till flower initiation in red beet (13 isoforms). Less isoforms are found in leaf peroxidase than in stem or root peroxidase. In all plants the number of isoforms reduced during gamete initiation. The synthesis of new isoforms with Rf 0.26–0.38 and Rf > 0.40 in red beet 'Kamuoliai 2' might mark the first processes of flowering induction.

Key words: cabbage, kohlrabi, red beet, flowering induction, generative development, peroxidase, isoenzymes

INTRODUCTION

Flowering initiation in plants is a whole complex of biochemical, physiological and morphological processes covering events from flowering stimulus formation in leaves and apical meristems during flowering induction until flowering and fertilization [1]. Such compounds as auxins and phenols take part in the quantititative regulation of flowering initiation processes. In the investigations of plant transfer to generative development, focus is laid on the study of functional activity of enzymes such as peroxidase, which take part in the metabolism of the abovementioned compouds [2, 3]. According to Montavon et al. [4], the change of peroxidase activity would be an early metabolical event, directly or indirectly associated to the overall process of flowering initiation.

The aim of this work was to investigate the dynamics of peroxidase isoenzymes in various organs of cabbage, kohlrabi and red beet during flowering induction in a growth chamber and the further generative development in the field.

MATERIALS AND METHODS

Trials were caried out with cabbage (*Brassica oleracea* L. convar. *capitata* var. *alba* L.) 'Early Golden Acre', kohlrabi (*Brassica oleracea* L. convar. *acephala* var. *gongylodes* L.) 'White Vienna' and red beet (*Beta vulgaris* L. convar. *vulgaris* var. *rubra* Buren.) 'Kamuoliai 2'. Seeds were sown into pots (volume 1 dm^3) filled with peat, in the greenhouse at a temperature of +18° ± 5 °C and 16 h photoperiod (additional irradiation by SON-T Agro 400 lamps).

Plants with 6–8 true leaves were transferred to growth chambers with a low temperature (+4° ± 1 °C) and 8 h irradiation with luminescent lamps for vernalization (20 plants of each species) for 60 days. Vernalized plants were transplanted into the field at the end of May – beginning of June. Stages of organogenesis were determined according to F. Kuperman [5].

Peroxidase isoenzymic spectra were investigated during flowering induction and generative development. Isoenzymes were extracted from leaves and stem in cabbage and kohlrabi, from leaves and roots in red beet at organogenesis stages II–IV or from leaves and stem at organogenesis stages V–IX by TRIS–GLYC (pH 8.3) buffer and studied by the electrophoresis method according to the modified V. Jaaska methodology [6]. Peroxidase isoforms were established by staining gel with o-dianisidine and brenzcatechine, supplemented with hydrogen peroxide. In the obtained electrophoregrams, the electrophoretic protein movement (Rf) of each line was measured.

RESULTS AND DISCUSSION

Peroxidase isoforms in cabbage were not numerous. The amount of isoenzymes in the cabbage stem was changing during ontogenesis from 3 to 5 (Fig. 1), three of them had their stable Rf. The isoform with the smallest molecular weight (Rf 0.38) had a wide line in the electrophoretic spectrum. The biggest amount of isoforms was detected at the juvenile stage, during flowering induction and evocation (III–IV of organogenesis stages). The amount of stem peroxidase isoforms decreased in the spectrum during flower initiation and gametogenesis (stages V–VIII). Lesser peroxidase isoforms were detected in cabbage leaves. One of them with Rf 0.38 was stable up to the flowering. During flowering, six isoforms of leaf peroxidase were noted. The new forms with a higher molecular weight and thus less active were synthesized at the Rf interval from 0.03 to 0.33. The isoform with Rf 0.46 had a wide line in the spectrum of flowering cabbage leaves.

The amount of peroxidase isoforms in kohlrabi in the course of ontogenesis was changing from 2 to 6 in leaves and from 3 to 7 per stem (Fig. 2). Stable isoenzymes in stem with Rf 0.18 and 0.37 had broad lines in the electrophoretic spectrum. At the end of evocation (stage IV) new lines appeared in the spectrum and this tendency survived during flower initiation (stage V). In this period new peroxidase lines with Rf 0.30 and 0.35 were distinguished in the stem spectrum. The amount of isoforms in kohlrabi leaves and stem decreased during gametogenesis (stages VI–VIII). The isoenzymic spectrum of leaf peroxidase changed totally during flowering: the amount of isoenzymes increased and the new forms with a lower molecular weight were synthesized. The form with Rf 0.48 had a wide line in the spectrum.

Peroxidase isoenzymic composition in red beet was more complicated. In the electrophoregrams of juvenile plants (stage II), 10–12 isoforms with a nearly even distribution from Rf 0.07 to Rf 0.56 were detected. Five lines had obscure lines or were detected only in some samples. Most isoforms with Rf < 0.40 had a distinct line.

During flowering induction of red beet the peroxidase isoenzymic spectrum changed, although changes of the apical dome were not visible. New isoforms with Rf > 0.40 were synthesized and their lines were distinct and narrow. These changes were more visible in the root peroxidase spectrum: there were eight of twelve isoforms with Rf $> > 0.40$, whose lines were distributed evenly close by each other. The tendency was observed during flowering induction, evocation and flower initiation. In this period four to six isoforms with a higher molecular weight were established with Rf < 0.40. Half of them had an obscure line in spectra. During flowering initiation one or two new peroxidase isoforms with Rf 0.26–0.38 were detected.

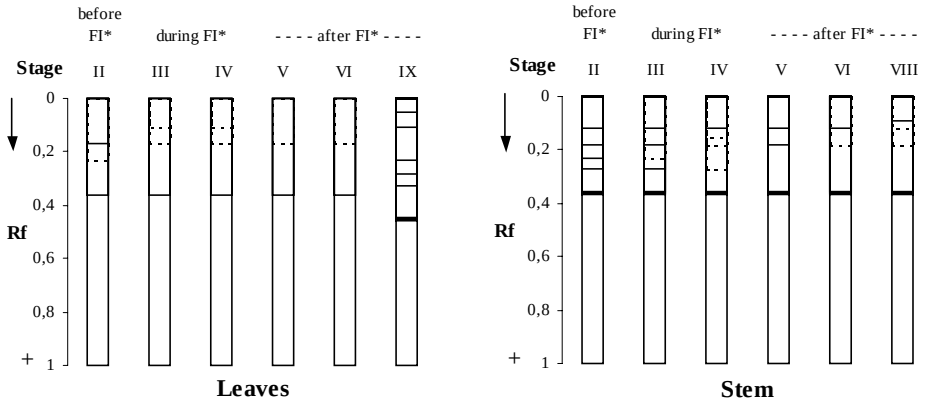

Fig. 1. Isoform spectra of leaf and stem peroxidase in cabbage 'Early Golden Acre' during flowering induction and generative development. FI* – flowering induction

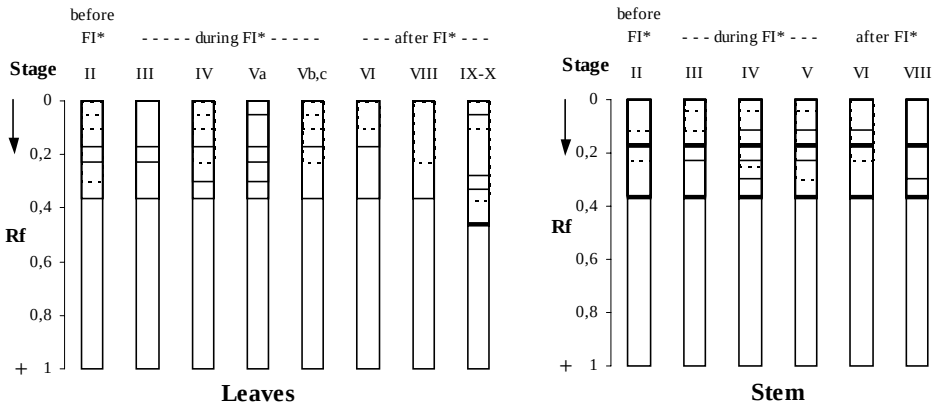

Fig. 2. Isoform spectra of leaf and stem peroxidase in kohlrabi 'White Vienna' during flowering induction and generative development. FI* – flowering induction

The obtained results on isoenzymic variation show that peroxidase of red beet 'Kamuoliai 2' is most active during flowering induction, evocation and flower initiation. The auxin (indole-3-acetic acid, IAA) in

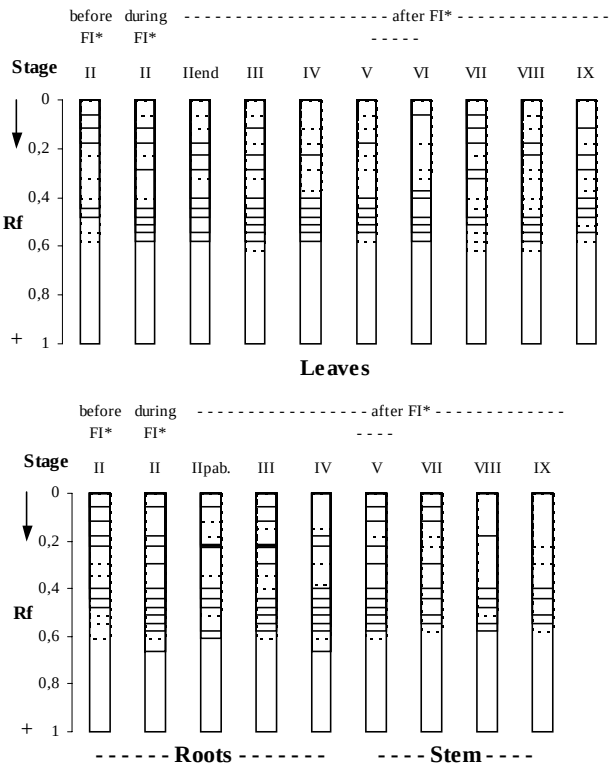

Fig. 3. Isoform spectra of leafes and stem peroxidase in red beet 'Kamuoliai 2' during flowering induction and generative development. FI* – flowering induction

which the metabolism of peroxidase takes part, binding with protein ABP participates in the processes of cell division and growth by elongation, root initiation, morphogenesis and organogenesis [7, 8], thus peroxidase is more active at the time of intensive organogenesis. The synthesis of new isoforms with Rf 0.26–0.38 and >0.40 might signify the first processes of flowering induction in red beet 'Kamuoliai 2'. During gemetogenesis up to flowering (stages VI–IX), the number of isoforms lowered, therefore it is possible to suppose that peroxidase had lost its vigor. Similar results were obtained with currant [3].

It was noted that isoenzymic spectra of leaf peroxidase within all species studied had less isoforms in comparison with stem or root peroxidase. This trend could be stipulated by differences in extensive growth of various tissues. It was established that the inductive transfer to flowering in spinach modulated the peroxidase activity differently in the roots and in the aerial part of the plant [4].

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GŪŽINIŲ, ROPINIŲ KOPŪSTŲ IR RAUDONŲJŲ BUROKĖLIŲ PEROKSIDAZĖS IZOFERMENTINIS SPEKTRAS ŽYDĖJIMO INDUKCIJOS IR GENERATYVINIO VYSTYMOSI METU

S a n t r a u k a

Tirta peroksidazės izofermentinės sudėties dinamika gūžinių kopūstų 'Early Golden Acre', ropinių kopūstų 'White Viena' ir raudonųjų burokėlių 'Kamuoliai 2' įvairiuose organuose, ontogenezėje. Gūžiniuose kopūstuose daugiausia fermento izoformų (5) rasta juvenaliniame tarpsnyje ir evokacijos metu, ropiniuose kopūstuose (7 izoformos) – evokacijos ir žiedų iniciacijos metu, burokėliuose (13 izoformų) – žydėjimo indukcijos metu, per evokaciją ir žiedų iniciaciją. Visų augalų lapuose užfiksuota mažiau peroksidazės izoformų negu stiebuose ar šaknyse. Naujų izofermentų sintezę Rf zonoje nuo 0.26 iki 0.38 ir >0.40 galima sieti su žydėjimo iniciacijos procesų pradžia burokėliuose. Gametų iniciacijos metu visų augalų peroksidazės izofermentiniuose spektruose sumažėjo izoformų skaičius.

Raktažodžiai: gūžiniai kopūstai, ropiniai kopūstai, raudonieji burokėliai, žydėjimo indukcija, generatyvinis vystymasis, peroksidazė, izofermentai