BIOLOGIJA. 2006. Nr. 2. P. 85–88
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Inflorescence structure and flower development control in spring rape (*Brassica napus* L.)

D. Kazlauskienė, V. Gavelienė,

L. Novickienė

Laboratory of Plant Physiology,
Institute of Botany,
Žaliųjų ežerų 49,
LT-08406 Vilnius, Lithuania
E-mail: danguole.kazlauskiene@botanika.lt

The growth, morphogenesis of the reproductive structure of the spring rape variety 'Mascot' and the impact of the auxin physiological analogue TA-12 on its flower formation, flowering time and duration have been investigated. After transition of the vegetative apex into the generative one, rape flower primordia were initiated in a spiral phylotactic pattern. TA-12 activated cell division in shoot apical meristem and initiated the development of lateral meristems. The auxin physiological analogue TA-12 induced the initiation of flower primordia formation, of the anatomical and morphological structures of flowers and siliquae, increased the number of rape siliquae and seed mass.

Key words: spring rape, auxin physiological analog, flower primordium, flowering, reproductive structures

INRTODUCTION

The reproductive success of plant varieties is often dependent on their flowering time being adapted to the environmental conditions [1]. This adaptation involves the regulation of flowering by environmental stimuli (such as temperature and day length) and application of growth regulators. In Europe and in Lithuania, rape (*Brassica napus* L.) is one of the basic producers of oil and proteins. Over the recent decade, rape oil has found an increasing use in biofuel production. Areas under rape are so far insufficient and the yields are low. The yields are low for a number of reasons: rape cultivation is a complicated process, the scientific knowledge of the formation and development of its generative organs and the possibility to modify these processes by growth regulators is scanty [2–5]. Also, the majority of rape varieties have been developed abroad and in Lithuanian climatic conditions they fail to fully realize their traits conditioned by the genotype and heritability, – the period of their flowering is prolonged and seeds at different developmental stages coexist at the same time on the plant. All these factors reflect on rape plant productivity and seed quality.

Increasing productivity and yield stability, as well as improving the efficiency of breeding programs, requires a better understanding of the morphological and physiological parameters that affect rape flowering onset and duration. The aim of the current work was to study the initiation process of the reproductive structure of spring rape, the influence of the auxin physiological analogue TA-12 on improving the development of inflorescence and floral meristems, flowers and siliquae formation.

MATERIALS AND METHODS

The test objects were the vegetative cone and intact plants of spring rape (*Brassica napus* L.) 'Mascot'. The effect of compound TA-12 on the growth, flowering and siliquae formation was studied in small field trials (2004–2005) at the Experimental Bases of the Institute of Botany. The test compound TA-12 (2 mM) was sprayed at an optimal concentration [6] of water solution taking 100 ml for each plot at the 2–3 true leaf unfolded stage. The plot area under rape was 4 m^2. The experiments were performed in four replications.

For anatomical and morphological investigations, vegetative cones were excised from 10 plants of each variant at the stages of 4–5, 5–6, 6–7 and 7–8 true leaves. The prepared samples were fixed in a formalin–acetic acid–alcohol (1:1:20) (FAA) mixture, dehydrated in a graded ethanol series, embedded in paraffin, and cut with a rotary microtome into 10–15 μm sections [7]. Serial longitudinal sections were stained with the periodic acid-Schiff's reagent (PAS) and photographed with a light microscope and a digital video camera (Olympus) (DP-11). The images were analyzed using the SigmaScan Pro (Jandel Scientific Software) program. The number of flowers in the terminal and lateral inflorescences was recorder just after the occurrence of the first anthesis, selecting the same plants 20 in each replication. The number of flowers and siliquae was counted every three days. The means values (M), ± standard error (SE) of the mentioned indices per plant were calculated as an average of 80 plants. The obtained data were treated statistically using the standard Excel 7 computer program.

RESULTS AND DISCUSSION

The shoot apical meristem (SAM) gives rise to the entire above-ground part, and its derivative meristems are responsible for the branches [8]. Inflorescence meristems are reproductive apical meristems that give rise to floral meristems. Apical meristems, including inflorescence meristems, are often indeterminate, maintaining their activity for an indefinite period of time. The flower meristem is determinate, having a finite duration and producing a definite number of organ primordia [9].

The anatomical analysis of rape shoot apex at the 3–4 leaf stage showed that in the control variant the shoot apex was in the vegetative stage and only leaf primordia were developed (Fig. 1A). At the same time, under the effect of TA-12 around the basal leaf axils the axillary buds were already formed (Fig. 1B). Morphometric measurements of apex width and height (from the top to the leaf primordium axis) showed that in the test variant the apex width was 150 ± 0.63 μm and height 75 ± 0.32 μm, *versus* 130±0.54 μm and 60±0.33 μm in the control plants.

Analyzing the developing rape vegetative cone at the 4–5 leaf stage, we see that both in the test and the control variants the apex is in the generative stage of development, however, plants exposed to the effect of TA-12 have already formed flower buds (Fig. 1D), whereas only axillary buds are formed in the control variant (Fig. 1C). It is possible that under the effect of TA-12 the apical meristem is determined earlier and thus gives rise to inflorescence meristems which in turn give rise to floral meristems. The monitoring of the development of the vegetative cone under the effect of TA-12 showed that at the 5–6 leaf stage peduncles, sepals, stamens and petal primordia were formed (Fig. 1F), whereas in control plants only peduncles and sepals were developed (Fig.1E). Flowers arose on the inflorescence apex in a phylotactic spiral pattern. This model is characteristic not only of the terminal inflorescence, but also of axillary branch inflorescences initiated on each leaf axis. In the subsequent stage (6–7 leaves), under the effect of TA-12 a fecundated ovule is seen in the ovary, together with seed buds and the initiated embryo development inside the endosperm (Fig. 1H), while in the control variant only seed-bud primordials are seen in the stage of formation (Fig. 1G).

Thus, the auxin physiological analogue TA-12 exerted a considerable effect on the development of spring rape generative organs and enhanced the processes of flowering and embryogenesis.

These data were substantiated and complemented with flower and siliquae formation monitoring data. Flowers and siliquae were counted in the terminal and axillary branch inflorescences, beginning with the opening of the first flower in the terminal inflorescence of the stem. The obtained data showed that under the effect of compound TA-12 the first flowers in the terminal inflorescence opened 3–4 days earlier. On the most intensive day of spring rape flowering (day 9), in the terminal inflorescence of test plants 10–11 flowers opened, *versus* 6–7 flowers in control plants (Fig. 2A). In the test plants, over the flowering period 36 flowers opened in the terminal inflorescences, of them 27 formed siliquae

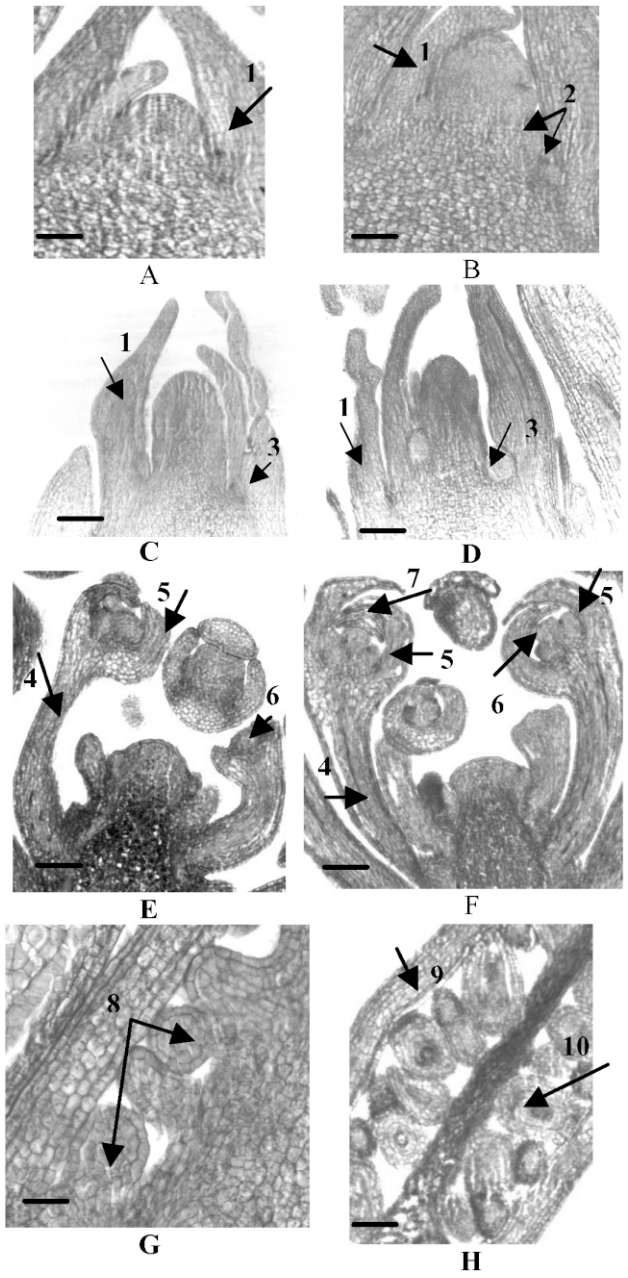

Fig. 1. Effect of TA-12 (2 mM) on spring rape 'Mascot' growth, organogenesis and embryogenesis: A, C, E, G – control variant; B, D, F, H – test variant:
(A) apex in the stage of vegetative development; **(B)** apex in the stage of generative development: leaves (1) and lateral branch primordia (2); (**C**) developed leaves (1) and axillary branches (2); (**D**) flower buds already formed (3); **(E)** developed pedicle (4), sepals (5) and stamens development initiation (6); **(F)** developed pedicle (4), sepals (5), stamens (6) and petals (7); **(G)** seedbud setting in the ovary (8); **(H)** fully developed seedbuds (10) in the ovary (9). Bars in A, B, C, D, E, F – 25 μm, in G, H – 50 μm

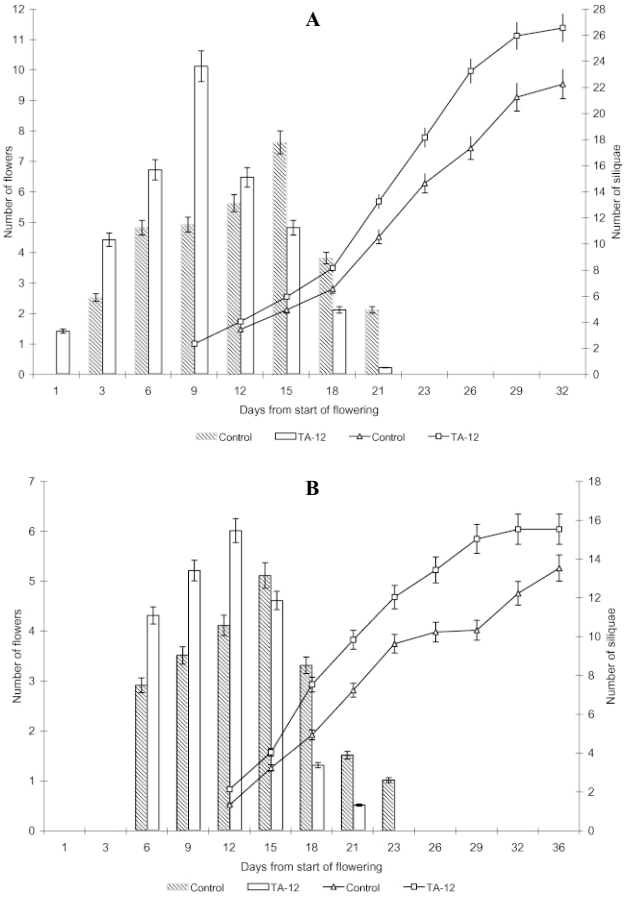

Fig.2. Effect of compound TA-12 (2 mM) on the spring rape 'Mascot' flowers and siliquae formation on the terminal (A) and lateral racemes (B)

and fully ripened seeds, *versus* 31 and 22, respectively, in control plants. The effect of TA-12 initiated an earlier flowering and thus an earlier formation of siliquae which assimilated a larger portion of nutritive substances as compared with siliquae of later settings.

According to data reported in the literature [10–12], 30–50 flowers are formed in the terminal inflorescence of the stem in spring rape, however, of them only about 70% form siliquae and ripen seeds.

We counted also flowers produced by axillary branches and found that in the control variant these buds opened on day 6 from the beginning of flowering, while rape plants exposed to TA-12 did it three days earlier. The maximum number of flowers opened on axillary branches was observed on day 12 of flowering, *versus* day 15 in control plants (Fig. 2B). Thus, TA-12 stimulated rape flowering both in the apical and axillary inflorescences; as a result, in these plants the flowering lasted 24–26 days, *versus* 29–31 days in control plants.

The potential number of siliquae in a plant is programmed rather early at the beginning of apex differentiation, apex axillary branch activation and at flower initiation [11, 12]. In our study, the first siliquae in the terminal inflorescence of the test variant began forming three days earlier than in the control variant (Fig. 2A). The greatest difference in the number of siliquae between the control and the test variant was observed on day 26 from the beginning of flowering: under the effect of the test compound 24 siliquae were formed, *versus* 17 in control plants (Fig 2A). As for the average number of siliquae per plant, the stimulating effect of TA-12 was obvious: the number of siliquae formed in the terminal inflorescence of the stem was by 11% and in axillary branches by 12% greater than in plants of the control variant. Our data are in agreement with the results which demonstrated that in vegetative experiments on spring rape 'Star' under the influence of compound TA-12, flowering in the terminal inflorescence started 3–4 days earlier and in axillary inflorescences 6 days earlier, with the earlier formation and growth of siliques and seeds [6].

Summarizing our data, it is possible to state that the physiological analogue of auxin compound, TA-12, has a considerable effect on the development and growth of spring rape generative organs. The anatomical and histological studies of the vegetative cone development showed that compound TA-12 modifies and stimulates the transition of the apex vegetative phase into generative one, the development of flower primordium up to the formation of anatomical and morphological flower structures. In small field trials, under the influence of compound TA-12, flowering in the terminal inflorescence started 3–4 days earlier and in axillary branches 3–6 days earlier, with the earlier formation and growth of siliquae and seeds.

ACKNOWLEDGEMENTS

This work was supported by the Lithuanian State Science and Studies Foundation.

Received 24 February 2006
Accepted 12 May 2006

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VASARINIŲ RAPSŲ (*BRASSICA NAPUS* L.) ŽIEDYNO STRUKTŪRA IR ŽIEDŲ VYSTYMOSI KONTROLĖ

Santrauka

Botanikos instituto Augalų fiziologijos laboratorijoje susiformavo eksperimentais pagrįsta nuomonė, kad augalų augimo procesus galima kontroliuoti norima linkme ne viso augalo, o atskirų organų atžvilgiu, panaudojant junginius, kurie yra ne fitohormonai, bet veikia kaip jų analogai.

Darbo tikslas – vasarinių rapsų ‘Mascot‘ augimo, morfogenezės, generatyvinio vystymosi, žiedų iniciacijos, žydėjimo trukmės ir ankštarų formavimosi kontrolė panaudojant auksino fiziologinį analogą TA-12.

Nustatyta, kad paveikus rapsus TA-12 junginiu (2 mM), 2–3 lapų vystymosi tarpsnyje buvo aktyvuojamas stiebo apikalinės meristemos ląstelių dalijimasis ir skatinamas vegetatyvinio vystymosi perėjimas į generatyvinį vystymosi tarpsnį. Auksino fiziologinis analogas, skatindamas lateralinių meristemų vystymąsi, indukavo žiedo užuomazgų formavimą, žiedų ir ankštarų anatominių bei morfologinių struktūrų susidarymą, sutrumpino žydėjimo trukmę, padidino augalo ankštarų skaičių.