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Role of endogenous growth regulators in vernalization of seeds of radish (*Raphanus sativus* L.)

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Abstract

In embryos and cotyledons of seeds of the radish cv. 'Tetra Iłówiecka' (which needs 20 days of vernalization) and cv. 'Saxa' (which flowers without vernalization) germinating at a vernalizing temperature of 5° C, the levels of auxins, gibberellins, cytokinins and the abscisic acid-like inhibitor were determined. The analyses were performed after 5, 10, 15, 20, 25 and 30 days of chilling. The levels of growth regulators were also determined in embryos and cotyledons of seeds germinated at 26° C when in the same growth stage as the material taken from chilled seeds. Cold treatment significantly affected the level of all endogenous growth regulators in embryos and cotyledons of both varieties. However, changes in the levels of these substances were not directly connected with the vernalization process. It was found that the vernalization of seeds of the radish cv. 'Tetra Iłówiecka' increased the level of GAs in leaves, this did not, however, coincide with flower initiation. It is concluded that the role of GAs in flowering of the studied plants is connected rather with photoinduction than with vernalization.

INTRODUCTION

The problem of the role of plant hormones in the vernalization process is as yet unsolved. According to many authors, vernalization provokes changes in the level of auxins, gibberellins, cytokinin and growth inhibitors (see Michniewicz et al. 1978). In our experiments with wheat (Michniewicz et al. 1978) it was found that the changes in the activity of auxins, cytokinins and the ABA-like inhibitor found in embryos following cold treatment were not directly connected with the vernalization process. However, we did not elucidate the question whether the changes in the level of gibberellins (GAs) which take place in embryos during thermoinduction are specific for vernalization. Thus,

we decided to repeat this study using another plant material — namely radish and to give more attention to the role of GAs in this process. Radish belongs to rosette plants, which are especially sensitive to GAs. In such plants vernalization can be fully or partially replaced by gibberellin treatment (Lang 1965).

MATERIAL AND METHODS

Basing on the results of preliminary experiments two varieties of radish were chosen: 'Tetra Iłówicka' which needs 20 days of seed vernalization and 'Saxa' which flowers without vernalization.

The seeds germinated for 40 hrs (cv. 'Tetra Iłówiecka') or 48 hrs (cv. 'Saxa') at 26° C were transferred to the vernalizing temperature of 5° C for a period of 5, 10, 15, 20, 25 or 30 days. The details of the vernalization procedure and the methods of analyses of plant hormones are given in the paper of Michniewicz et al. (1978).

Plant hormones: auxins, GAs, cytokinins and the ABA-like inhibitor were determined in the embryos and cotyledons after 40 or 48 hrs of germination at 26° C followed by 5, 10, 15, 20, 25 and 30 days of chilling. The analyses were performed also in the embryos and cotyledons of seeds germinated at 26° C which reached the same growth stage as the chilled material (Table 1).

Table 1

Time of seed germination at 26°C required for the attainment of a growth stage corresponding to that attained on a given day of vernalization

Days of seed vernalization at 5°C	Hours of seed germination at 26°C	
	cv. 'Tetra Iłówiecka'	cv. 'Saxa'
0	40	48
5	44	79
10	48	96
15	72	120
20	86	144
25	120	173
30	144	necrosis

In a special experiment the levels of GAs in cotyledons and leaves of radish plants cv. 'Tetra Iłówiecka' grown from vernalized and unvernalized seeds were analysed. For analyses 50-g samples were taken.

The results of preliminary experiments showed that the plants flower only in a long photoperiod. When cultivated under short day conditions (8 hrs) they do not flower despite vernalization of seed. It was

also found that the plants remain in juvenile state till the development of 9 leaf primordia (Zatorska, unpublished). Seeds vernalized for 20 days were sown into containers with garden soil and cultivated on a 16-hr day at a temperature of 26° C and a light intensity of about 6000 lx.

All experiments were replicated 3 times and the results were evaluated statistically with the method of analysis of Student's t-test by estimating LSD at $P = 0.05$.

RESULTS

EXPERIMENT I. EFFECT OF VERNALIZING TEMPERATURE ON THE LEVEL OF PLANT HORMONES IN EMBRYOS AND COTYLEDONS OF RADISH CV. 'TETRA IŁÓWIECKA'

During the cold treatment the level of all tested plant hormones markedly increased. The level of auxins (Fig. 1) and of the ABA-like inhibitor (Fig. 2) reached the highest values after 30 days of chilling. However, the highest values of gibberellins (Fig. 2) and cytokinins (Fig. 1) were found after 20 and 15 days, respectively.

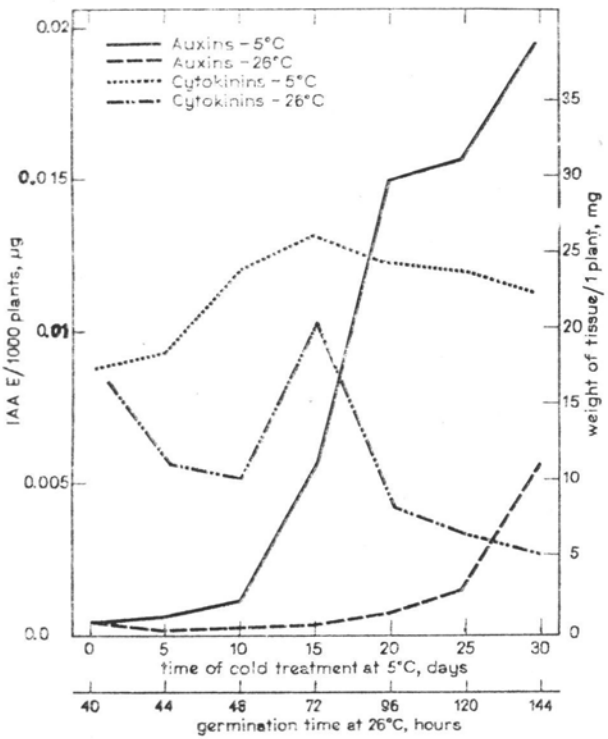

Fig. 1. The dynamics of auxins and cytokinins in embryos and cotyledons of seeds of radish cv. 'Tetra Iłówiecka' during germination at a vernalizing temperature 5° C and at 26° C

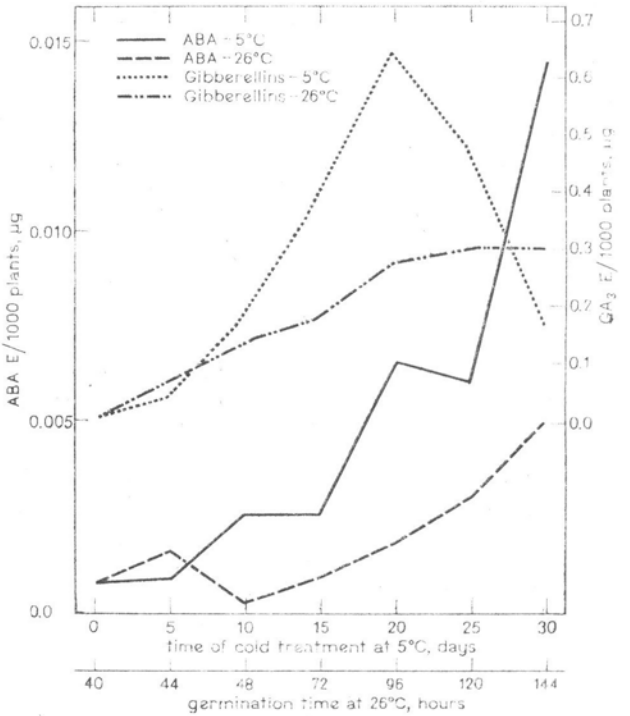

Fig. 2. The dynamics of GAs and ABA-like inhibitor in embryos and cotyledons of seeds of radish cv. 'Tetra Iłówiecka' during germination at a vernalizing temperature 5° C and at 26° C

These figures allow us to compare the activity of plant hormones in tissues taken from chilled and unchilled seeds when in the same growth stage. This comparison permits the conclusion that chilling significantly increases the level of all plant hormones tested. Only the level of GAs in embryos and cotyledons of seeds chilled 30 days was lower.

EXPERIMENT II. EFFECT OF VERNALIZING TEMPERATURE ON THE LEVEL OF PLANT HORMONES IN EMBRYOS AND COTYLEDONS OF RADISH CV. 'SAXA'

Similarly as in the experiment with radish cv. 'Tetra Iłówiecka' it is clear that during germination of seeds at 5° C (thermoinduction) the level of auxins (Fig. 3), GAs (Fig. 4) and cytokinins (Fig. 3) increased. However, contrary to the results with 'Tetra Iłówiecka' the level of the ABA-like inhibitor (Fig. 4) markedly decreased.

From comparison of the activity of growth regulators in plant material taken from vernalized and unvernalized seeds when in the same growth stage it is also evident that similarly as in the case of radish

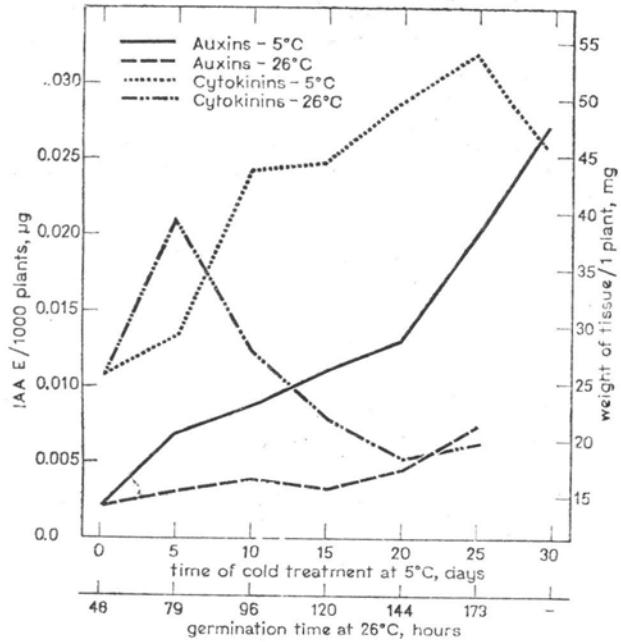

Fig. 3. The dynamics of auxins and cytokinins in embryos and cotyledons of seeds of radish cv. 'Saxa' during germination at a vernalizing temperature 5° C and at 26° C

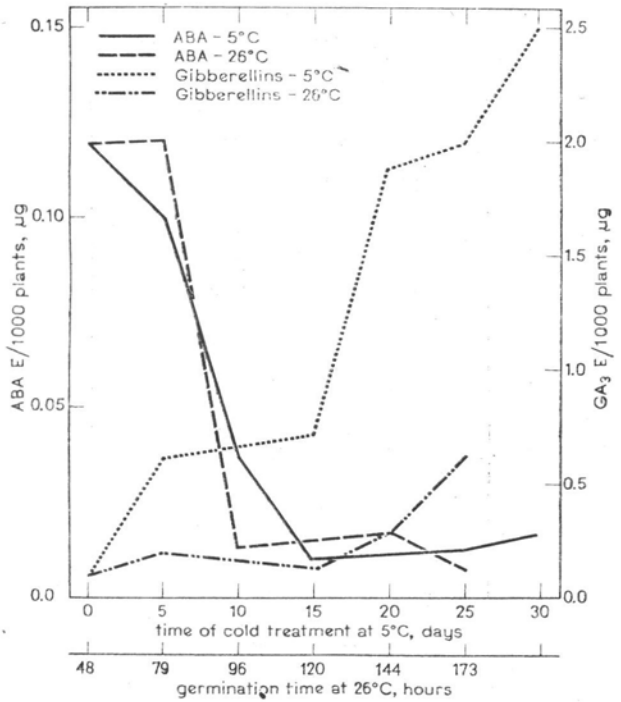

Fig. 4. The dynamics of GAs and ABA-like inhibitor in embryos and cotyledons of seeds of radish cv. 'Saxa' during germination at a vernalizing temperature of 5° C and at 26° C

cv. 'Tetra Iłówiecka' chilling significantly increased the level of auxins GAs and cytokinins. Only the effect of chilling on the level of the ABA--like inhibitor was obviously less at least in cv. 'Saxa'.

EXPERIMENT III. EFFECT OF VERNALIZATION OF SEEDS ON THE LEVEL OF GAS IN COTYLEDONS AND LEAVES IN RADISH PLANTS CV. 'TETRA IŁÓWIECKA'

Vernalization of seeds stimulated the elongation of plants and in some degree also the development of leaves (Table 2).

Table 2

Effect of seed vernalization on growth and development of radish cv. 'Tetra Iłówiecka'

Time of cultivation (days)	Height of plants (cm)		Number of leaves	
	unvernalized	vernalized	unvernalized	vernalized
7	3.8	2.8	cotyledons	cotyledons
14	4.5	5.6	2-3	2-3
21	7.2	10.7	3-4	3-4
28	10.7	13.5	4	4-5

The level of GAs in cotyledons and leaves depended on the age of the plants. The level of these compounds increased during plant growth and reached the highest value on the 21 day of plant growth and then decreased (Fig. 5). The samples taken for analyses from vernalized plants were characterized by a higher level of GAs in comparison to samples taken from plants raised from unvernalized seeds.

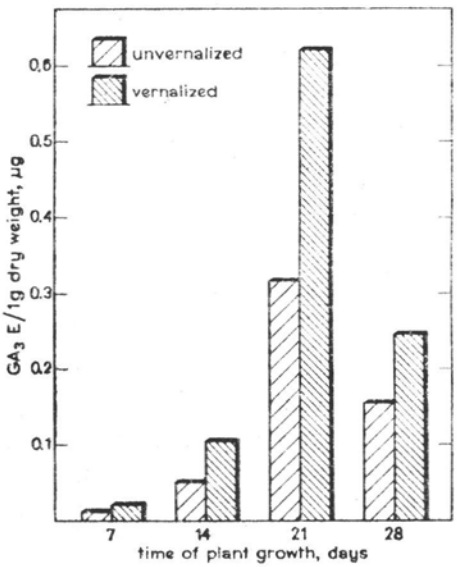

Fig. 5. Effect of seed vernalization on the level of GAs in cotyledons (7 days) and leaves of radish cv. 'Tetra Iłówiecka'

DISCUSSION

From the results of experiments I and II it is clear that (similarly as in experiments with wheat) cold treatment provokes marked changes in the level of all determined plants hormones in embryos and cotyledons of both cultivars of radish: 'Tetra Iłówiecka' requiring vernalization and 'Saxa' which flowers without thermoinduction.

The comparison of the activity of plant hormones in material taken from chilled and unchilled seeds when in the same growth stages excludes the possibility that the observed changes in the level of the analysed substances depended on developmental changes taking place during germination at a vernalizing temperature. Thus, it is clear that the changes in the levels of plant hormones in response to cold treatment are not specific for vernalization.

This conclusion concerning auxins, cytokinins and the ABA-like inhibitor is in accordance with the results of our experiments with wheat. It does not, however, support our earlier suggestion that the increase in the level of GAs which takes place in embryos during thermoinduction may be specific for vernalization (Michniewicz et al. 1978).

Data supporting the view that changes in the level of GAs in seeds observed following chilling, are not specific for vernalization were also obtained by Günther (1966). He found that wheat kernels treated during thermoinduction with CCC, which inhibits biosynthesis of GAs, were inhibited in development both the winter and spring varieties. It is also known that chilling increases the level of GAs during the process of seed stratification (for review see Taylorson and Hendrics 1977) although this process does not coincide with flower initiation.

Some information concerning the role of GAs during seed vernalization was obtained from the results of analyses presented in Figs. 6 and 7. They show that in embryos and cotyledons of both radish cultivars taken from chilled and unchilled seeds when in the same growth stages, similar 3 groups of GA-like substances localized at R_f 0.0-0.3, 0.4-0.6 and 0.7-1.0 were found. Thus, these results were in accordance with our data obtained in experiments with wheat (Michniewicz et al. 1978) and suggest that during seed chilling no qualitative changes in GAs take place.

This observation is also in accordance with the results of Harris and Atherton (1976) who in experiments with *Dianthus barbatus* did not find any evidences that chilling brought about the production of different GAs. From the results obtained in experiments with *Hyoscyamus niger* (Reinhard and Lang 1961) and olive (Badr et al. 1970) it is also evident that "new" GAs appeared in extracts of cold-requiring plants only shortly before flower initiation.

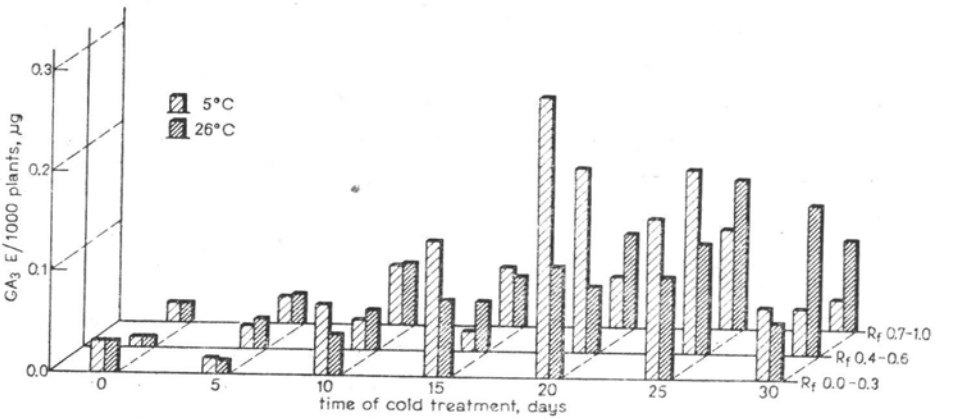

Fig. 6. Chromatographic analysis of GAs isolated from embryos and cotyledons of seeds of radish cv. 'Tetra Iłówiecka'

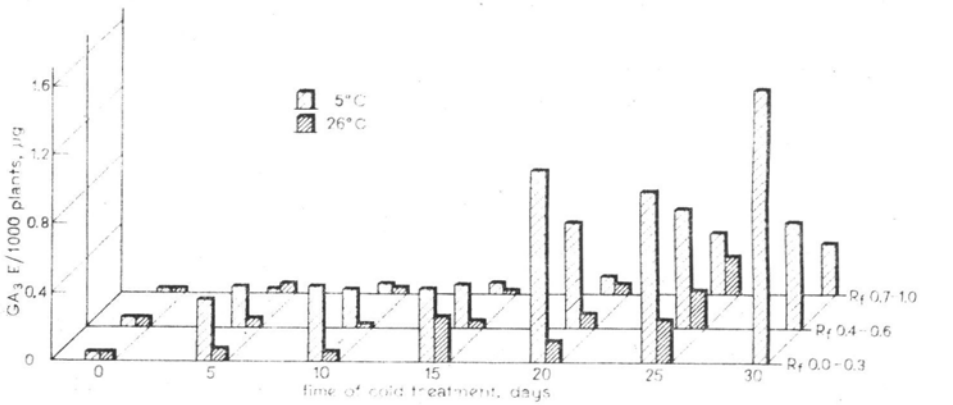

Fig. 7. Chromatographic analysis of GAs isolated from embryos and cotyledons of seeds of radish cv. 'Saxa'

Some information concerning the role of GAs in the vernalization process was obtained in experiment III. It was shown that the leaves of plants raised from vernalized seeds contained more GAs as compared with unvernalized plants. A similar effect of seed vernalization on the level of GAs in radish plants was found by Suge and Rappaport (1968) and Suge (1970), and wheat plants by El-Antably (1976). However, in our experiments it was shown that after 28 days of plant growth, when the plants were still in the vegetative stage of development (conclusion based on unpublished results of Zatorska) the level of GAs in vernalized as well as in unvernalized plants markedly decreased.

Taking into consideration the fact that flower initiation takes place later than after 28 days of growth, it must be concluded that the in-

crease of GAs in leaves following seed vernalization does not coincide with flower initiation. It seems that this increase of endogenous GA-like activity was rather related to stem elongation (Table 2).

This conclusion would be in accordance with the results of experiments with other rosette plants — radish cv. 'Miyashige-sofuto' (Suge and Rappaport 1968, Suge 1970) and carrots (Hiller et al. 1979). This is also in agreement with the results of other authors cited by Hiller et al. (1979).

Radish cv. 'Tetra Iłowiecka' belongs to long-day plants, and according to our experiments, vernalized plants do not flower in short day conditions (8 hr). There are also some data in the literature showing that plants grown under long day contain more GAs than those grown under short day (for review, see Zeevaart 1976).

Taking all these facts into consideration it may be concluded that the role of GAs in the flowering of radish is connected rather with photoinduction than with vernalization.

Acknowledgments

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Studia nad rolą endogennych regulatorów wzrostu roślin w procesie wernalizacji nasion rzodkiewki (Raphanus sativus L.)

Streszczenie

Oznaczano poziom auksyn, giberelin, cytokinin i inhibitora o właściwościach kwasu abscysynowego w embrionach i liścieniach rzodkiewki odmiany 'Tetra Iłowiecka' (wymagającej 20-dniowej jaryzacji) i odmiany szklarniowej 'Saxa' (która zakwita bez jaryzacji) uzyskanych z nasion poddanych chłodzeniu w temperaturze 5° C. Analizy wykonywano po 5, 10, 15, 20, 25 i 30 dniach chłodzenia. Oznaczano także poziom tych substancji w embrionach i liścieniach pochodzących z nasion kiełkujących w temperaturze 26°C, które osiągnęły taką samą fazę wzrostu jak embriony pochodzące z nasion poddanych termoindukcji. Stwierdzono, że chłodzenie wywołuje istotne zmiany w poziomie wszystkich badanych fitohormonów, jednakże zmiany te nie mają bezpośredniego związku z procesem wernalizacji. Stwierdzono także, że wernalizacja nasion rzodkiewki odmiany 'Tetra Iłowiecka' wyowołuje zwiększenie poziomu GAs w liściach roślin. To zwiększenie poziomu GAs będące efektem wernalizacji nasion nie ma jednak bezpośredniego związku z inicjacją kwitnienia. Wnioskuje się, że rola GAs w procesach prowadzących do przejścia badanych roślin do fazy generatywnej wiąże się raczej z fotoindukcją aniżeli z procesem wernalizacji.