*BULG. J. PLANT PHYSIOL., 1995, 21(1), 36–44*

# EFFECT OF GROWTH RETARDANTS ALAR AND MEIA ON PEROXIDASE AND IAA-OXIDASE ACTIVITY IN STEMS OF TOBACCO SEEDLINGS

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*Received April 19, 1995*

**Summary**. Plant growth retardants Alar (dimethylhydrazide of succinic acid) and its structural analogue MEIA (β-monomethyl ester of itaconic acid) at a concentration 2000 mg/l sprayed at the fourth true leaf stage of tobacco seedlings caused identical retardation in stem growth from the second till the tenth day after treatment. Soluble peroxidase activity in stems of the treated plants increased on the second day after treatment and remained the same until the end of the investigated period. IAA-oxidase activity also increased on the second day after treatment, but later dropped slowly and reached the control level. Alar and MEIA did not influence peroxidase and IAA-oxidase activity *in vitro*. From the second day till the end of the investigated period an increase in total phenols and in the level of chlorogenic acid in the stems of the treated plants was also observed. The increase of IAA-oxidase activity post treatment with growth retardants Alar and MEIA probably took part in the regulation of stem growth of tobacco seedlings. The regulation of enzyme activity might not be direct and could be due to the participation of phenol compounds.

***Key words***: retardants, peroxidase, phenols

***Abbreviation***: Alar – N,N-dimethylhydrazide of succinic acid; MEIA – β-monomethyl ester of itaconic acid; IAA – indolyl-3-acetic acid

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## Introduction

Alar is a growth retardant with proven effect and practical application on many plant species (Wittwer, 1971). However, its use in recent years has been limited because of data about its toxic effect (Hurter et al., 1988). MEIA is a growth retardant with higher activity than Alar in retarding the growth of pea plants and cucumber hypocotyls, with low $LD_{50}$=500 mg/kg live weight in Agness Blum albino mice test (Karanov et al., 1975). Both growth retardants have a similar chemical structure, which could mean also similar mechanisms of action. An equally retarded growth rate was established in stems of tobacco seedlings ten days post their treatment with various Alar and MEIA concentrations (Petkova and Angelova, 1994). It is a known fact that gibberellin and auxin phytohormones play a significant role in the process of stem elongation (Graebe, 1987). Lower gibberellin content after plant treatment with Alar is not due to blocking of biosynthesis (Muromtzev et al., 1989), but most probably to blocking its transformation into active forms (Menhenett, 1981), the transport to intensively growing zones, as well as to enhanced conjugation into glycoside and glycosil-esters (Taceno, 1981). Auxin content is also reduced after plant treatment with Alar (Jindal et al., 1977) due most probably to the change in enzyme activities from the biosynthetic (Reed et al., 1965) or the catabolic pathway (Jain et al., 1969). Basic peroxidases affect by their oxidase function the deactivation of auxins by degrading them(Gaspar et al., 1985). In a relation with this the present work is an attempt to follow in dynamics the growth retarding effect of Alar and MEIA on tobacco seedlings, as well as the changes in soluble peroxidase and IAA-oxidase activities. Changes in the amounts of total phenols and of chlorogenic acid, the inhibitor of IAA-oxidase activity (Chirek, 1990) were also investigated.

## Material and Methods

The investigation were carried out with tobacco of oriental type (*Nicotiana tabacum* cv. Plovdiv 5) grown in glasshouse conditions. Vegetation pots with a diameter of 25 cm, filled with soil and manure in a 2:1 ratio, were used in which 0.8 g/$m^2$ seeds were sown. The treatment with Alar and MEIA in concentration 2 000 mg/$dm^3$ was made by spraying the plants in one pot with 12 ml of the respective solutions. The treated plants were in the fourth true leaf stage. Samples for analysis were taken in the day of treatment and on the second, fourth, seventh and tenth day after treatment.

Peroxidase activity was determined according Herzog and Fahimi (1973). One gram plant material was ground in 5 ml of 0.05 M phosphate buffer (pH 6.1) and centrifugated for 30 min at 16 000 g . The reaction medium used for assaying enzyme activity contained 10 µl of enzyme extract; 25 µl of 0.6% $H_2O_2$ and 1.35 ml of 3,3-diaminobenzidine solution as hydrogen donor. The changes of optical density were registered at 465 nm.

IAA-oxidase activity was determined after Gamburg (1966). Two and a half grams of plant material were ground in 5 ml of 0.02 M phosphate buffer (pH 6.1) and centrifugated for 30 min at 16 000 g. The supernatant was purified through a column with Sephadex G-50. The collected three fractions of 2 ml each with highest protein and enzyme activity were used in determining IAA-oxidase activity. The reaction medium used in enzyme activity determination contained 0.5 ml of enzyme extract; 2 ml of $10^{-3}$ M IAA; 1 ml of $10^{-3}$ M $MnCl_2$, 1 ml of $10^{-3}$ M 2,4-dichlorophenol and 5.5 ml of 0.02 M phosphate buffer (pH 6.1). Alar and MEIA were added in reaction medium in the experiments *in vitro.* Forty min reaction time was established in our previous experiment as optimal. The changes in IAA quantity were registered at 530 nm after one hour reaction with Salkovski's reactive.

The amounts of total phenols was determined after the method of Swein and Hillis (1959). Total phenols were extracted from the plant material with ethanol. The reaction medium used in determining the amounts of total phenols contained 0.5 ml of ethanolic extract, 0.5 ml of Folin reagent and 1 ml of $Na_2CO_3$. The changes in optical density were registered at 730 nm. The results were calculated after the standard curve with chlorogenic acid.

The content of chlorogenic acid was determined after Hausermann and Waltz (1962). The reaction medium used in determining chlorogenic acid contained 1 ml of ethanolic extract; 0.5 ml of 0.5 M phosphate buffer and 0.2 ml of 1% flavognost solution. The changes in optical density were measured at 381 nm.

Total soluble protein content was estimated after the method of Lowry et al. (1951).

## Results

Results of the stem growth inhibition of tobacco seedlings in dynamics after Alar and MEIA treatment (concentration 2000 mg/dm$^3$) are presented in Fig. 1. Growth retardation in Alar treated plants began on the second day after treatment, increased gradually and on the seventh and tenth day their stems were about 30% shorter than the stems of control plants. In MEIA treated seedlings the effect was less pronounced on the second and fourth day, but on the seventh and tenth day it was close to that of Alar.

The results of the soluble peroxidase activity changes in the stems of tobacco plants are presented in Fig. 2*A*. On the second day after Alar treatment of the seedlings, soluble peroxidase activity in their stems was considerably increased and with small fluctuations kept its level up to the tenth day. In stems of MEIA treated seedlings an increase of soluble peroxidase was also observed, but it was much smaller than in the stems of Alar treated seedlings. Ten days post treatment the enzyme activity in stems of Alar and MEIA treated seedlings was the same.

Results of the IAA-oxidase activity changes in the stems of tobacco plants are shown in Fig. 2*B*. A considerable increase in the enzyme activity was observed on

**Fig. 1.** The influence of Alar and MEIA on the length of the stems of tobacco plants

the second day after Alar and MEIA treatment. On the fourth and seventh day the differences in IAA-oxidase activity in stems of control and treated seedlings were gradually reduced and on the tenth day they did not exist.

Results of *in vitro* conducted experiments with Alar and MEIA in $10^{-9}$M to $10^{-5}$M concentrations concerning their direct influence on soluble peroxidase and IAA-oxidase activities are presented in Table 1. Data show that Alar and MEIA in applied concentrations did not affect *in vitro* the activity of both enzymes.

**Table 1.** The influence of Alar and MEIA on the activity of peroxidase and IAA-oxidase from tobacco stems *in vitro*

| | Peroxidase units per ml | | μg degraded IAA mg protein$^{-1}$min$^{-1}$ | |
|---|---|---|---|---|
| Concentration | Alar | MEIA | Alar | MEIA |
| $10^{-5}$ M | 14.6±0.6 | 17.0±2.0 | 66.0±12.0 | 78.8±19.2 |
| $10^{-6}$ M | 14.5±1.6 | 16.0±1.0 | 71.2±11.2 | 77.3±16.0 |
| $10^{-7}$ M | 12.9±0.8 | 15.5±2.0 | 73.7±11.9 | 78.1±18.4 |
| $10^{-8}$ M | 13.8±1.1 | 16.8±2.9 | 71.4±10.8 | 80.0±16.2 |
| $10^{-9}$ M | 14.7±2.0 | 17.4±3.0 | 74.2±12.9 | 83.0±16.2 |
| Control | 13.2±1.4 | 17.4±2.8 | 66.2±12.0 | 73.4±19.2 |

**Fig. 2.** The influence of Alar and MEIA on the peroxidase (*A*) and IAA-oxidase (*B*) activity in the stems of tobacco plants

Results about total phenol changes in tobacco stems after Alar and MEIA treatment are presented on Fig. 3*A*. The amounts of total phenols increased considerably

on the second day after Alar treatment and continued to rise up to the tenth day. In the stems of MEIA treated seedlings an increase of total phenol amounts was also

**Fig. 3.** The influence of Alar and MEIA on the amounts of soluble phenols (*A*) and chlorogenic acid (*B*) in the stems of tobacco plants

observed, but it was much lower than that in Alar treated seedlings. The content of chlorogenic acid, which is the main phenol inhibitor of IAA-oxidase activity and a widely extended phenol in tobacco plants (Loche, 1966) changed also after Alar and MEIA plant treatment. Results concerning the changes of chlorogenic acid content are shown in Fig. 3*B*. The stems of Alar and MEIA treated plants had higher content of chlorogenic acid than the control ones on all days of the investigation except on the fourth day.

## Discussion

Alar applied directly to the leaves or through the roots, retards stem growth of tobacco plants like other growth retardants (Kado et al., 1969; Pandey, 1973). Applied on the leaves Alar is actively absorbed and transferred to plant roots in the first three days post treatment (Zeevaart, 1966; Dick, 1972). Our experiments conducted in dynamics showed that on the second and fourth days after Alar and MEIA tobacco treatment was observed a slight retardation of stem growth, which was gradually enhanced and was well expressed on the seventh and tenth day. The changes in peroxidase and IAA-oxidase activity, total phenols and chlorogenic acid contents were clearly manifested on the second day and they probably take part in the following considerable growth inhibition.

It is a well known fact that peroxidase can regulate plant stem growth by changing the endogenic auxin content (Gaspar et al., 1985), the oxidation of which is performed by its IAA-oxidase function. Our investigations showed a considerable increase both of peroxidase and IAA-oxidase activities in the stems of Alar and MEIA treated plants as early as the second day. In the following days IAA-oxidase activity was quickly reduced and came close to that of the control on the tenth day after treatment. At the same time the peroxidase activity remained significantly higher. This result can be explained by the suggestion that only a part of peroxidase izozymes manifest IAA-oxidase activity (Gove et al., 1975). Dencheva and Klisourska (1988) have established that retardant CCC affects in different ways the activity of cationic peroxidase izozymes of maize seedlings and especially izozyme N6 and its IAA-oxidase activity. Similar result showing different degree of increased IAA-oxidase and peroxidase activity was observed by Gaspar et al. (1968) in barley plant stems post treatment with the retardants CCC and AMO-1618. The higher peroxidase activity maybe takes part in the faster process of lignification after Alar and MEIA treatment (Petkova and Angelova, 1994). Results from our recent work showed an increase of total phenol amounts which maybe used as substrata of lignification. Alar and MEIA did not affect *in vitro* the activity of both enzymes, although there are reference data about activating IAA-oxidation by dixarboxyllic acids, such as oxalic, citric and malonic acids (Forchetti et al., 1983). The level of phenols, IAA-oxidase

activity and auxin level are closely connected and represent an important element of the growth regulating mechanism (Chirek, 1990). The retarded growth of tobacco seedling stems after Alar and MEIA treatment was preceded by a considerable increase of IAA-oxidase activity, the following decrease of which was probably due to a rise in the level of inhibitors of phenolic nature. Results of the present investigation showed the higher activity of Alar.

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