GROWTH CHARACTERISTICS OF GARLIC IN *IN VITRO* CONDITIONS*

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The growth characteristics of bolting garlic genotype 'Djambul2' were studied in *in vitro* conditions. The growth of the plants was described by parameters of the Mitscherlich logistic growth curve every week. Correlations among parameters characterized logistic growth were of physiological and mathematical shapes of the logistic curve. The characteristics of leaf area (A), dry weight of leaves (W) and leaf area duration (LAD) were positively correlated and they were associated with increasing trends. Changes in leaf area ratio (LAR) were 8.8% in a period of six weeks. The growth of leaves was compensated by lower net assimilation rates (NAR) that decreased to 6.6% over a period of six weeks. The production rate (C) significantly decreased to 42% after six weeks. The relative growth rate of dry weight (RGR RW) decreased to 5.8%, relative growth rate of assimilation area (RGR RA) reduced to 12.6%. The findings support the hypothesis about limitation of nutrition resources in growing media.

Allium sp.; tissue culture; leaf area ratio; production rate; net assimilation rate

INTRODUCTION

Plants, during their life cycle, are exposed to changing environmental conditions which can have a negative impact on their life functions, causing slow metabolism, damaged plant organs or, in extreme cases, leading to the death of entire plants. Garlic grown in field conditions has often a relatively high risk of disease infection (viral, bacterial and fungal), pests (nematodes, mites, thrips), degeneration and weakened plants as a result of continuous vegetative propagation. It is possible to solve the problem of preservation and propagation of plant material in *in vitro* conditions. The cultivation of plants by meristems allows to grow the plants without all the above mentioned infections and after virus eradication also as virus-free lines. For plant biodiversity maintenance and healthy plant material storage, it is suitable to use the plant tissue cultures (Ayabe, Sumi, 2001; Engelmann, 2009). The growth and regeneration of garlic (*Allium sativum* L.) in *in vitro* conditions may be affected by many factors. The rate of growth and dry matter production are primarily affected by physical factors, namely temperature, light intensity, humidity, abiotic chemical factors, and particularly the composition of the culture medium.

The plants of garlic come from Central Asia and require an annual cycle of temperature, warm-cold-warm, with inter-specific differences in the stage of the degree of cold hardiness. The species at the area of origin require a cold period of 4 to 17 °C for 4–16 weeks. Temperatures from 5 to 15 °C are needed for germination. It begins to increase the shoot tip, which is extended to create the first root, the first leaves grew from the ground after 4 month. At the end of the season, the bulbs are formed, while roots and leaves die out. The bulb increases in volume the next year. They also create non-fertile flowers with leaves and bulbs with cloves in the following year (Kamenetsky, 1994). The bulb formation in *in vitro* conditions can be affected not only by plant hormones, but also by the light conditions (day length), content of sucrose in the cultivation medium, as well as the temperature during cultivation (Le Guen-Le Saos et al., 2002).

The aim of this study was to determine the optimal growth condition for *in vitro* growth of garlic plants. Although there are a few reports related to *in vitro* propagation of garlic plants by shoot tips (Ayabe, Sumi, 2001; Engelmann, 2009; Wu et al., 2009), but all these studies have usually no results about growth characteristics. These characteristics can be useful in multiplication and micropropagation protocols for the production of genetically uniform garlic plants.

* Supported by the Ministry of Agriculture of the Czech Republic, Projects No. MZE0002700604, QH71228 and by IGA (Internal Grant Agency – Faculty of Agrobiology, Food and Natural Resources, Project No. 21110/1312/3111.

MATERIALS AND METHODS

The bolting genotype of garlic (*Allium sativum* L.) genotype 'Djambul2' was used for this experiment. 'Djambul2' is a landrace genotype originating from the former Soviet Union. The weight of 100 fresh bulbils was 6.9 ± 0.34 g (mean ± SD). Shoot tips of this genotype was excised, and surface sterilized with 2% solution of Parmetol (isothiazolinone, Schülke & Mayr GmbH, Norderstedt, Germany) for 20 minutes. Meristems were cultured on MS medium (Murashige, Skoog, 1962) supplemented with 0.2 mg l^{-1} BAP, 0.02 mg l^{-1} NAA, 30 g l^{-1} sucrose, 8 g l^{-1} agar, pH 5.7. Temperature at 20 ± 1°C and light intensity of 100 µE m^{-2} s^{-1} with 16-hr photoperiod were used during the culture. After six weeks of culturing, the plants were used as explants for the following experiments. Each experiment was replicated three times with 10 plants per variant.

Dry weight

Explants were cut and transferred onto a fresh MS culture medium and were cultivated in the same conditions for six weeks. The length of individual plants was measured from the base to the tip of the leaf every week. The dry weight of leaves (W) and leaf area (A) of garlic plants cultivated in *in vitro* conditions were determined. The plant material was dried in an oven at 85 °C until a constant weight was achieved.

Growth characteristics

Explants of garlic genotype 'Djambul2' grown in *in vitro* conditions were observed for six weeks. It was the optimal time period for garlic plants subcultivation *in vitro* according to preliminary experiments. At the collection of plant material, the fresh weight of the whole plants was checked every week. The leaves of garlic were scanned and the leaf area (A) was then determined using an image analysis software ImageJ (ImageJ, National Institutes of Health, USA). The raw images were converted to 24-bit bitmap format, via gray scale transformated to the black-and-white binary images (1-white, 0-black). According to the formulas (see below) to determine the growth characteristics, the leaf area ratio (LAR), the leaf area duration (LAD), the production rate (C), the relative growth rate of dry weight (RGR RW), the relative growth rate of assimilation area (RGR RA) and the net assimilation rate (NAR) were calculated.

The formulas for calculating the growth characteristics were used according to the plant growth analysis by Evans (1972); Hunt et al., (2002) and Lambers et al., (2008).

$$LAR = A/W \quad (1)$$

$$LAD = [(A_2 - A_1)/(\ln A_2 - \ln A_1)]\ (t_2 - t_1) \quad (2)$$

$$C = [(W_2 - W_1)/(t_2 - t_1)]\ 1/P \quad (3)$$

$$RGR\ RW = (\ln W_2 - \ln W_1)/(t_2 - t_1) \quad (4)$$

$$RGR\ RA = (\ln A_2 - \ln A_1)/(t_2 - t_1) \quad (5)$$

$$NAR = [(\ln A_2 - \ln A_1)/(t_2 - t_1)]\ [(W_2 - W_1)/(A_2 - A_1)] \quad (6)$$

where:
A is leaf area,
W is dry weight,
P is ground area,
t is time in days,
index 1 is the value of the first sampling,
index 2 is the value of the next sampling.

Statistical analysis

Measured data were analyzed by Mitscherlich's growth function (Mitscherlich, 1928):

$$y = A\ (-1 + \exp^{(-R\ t)}) \quad (7)$$

that was characterized by no inflection with asymptote at $f(t) = A$ (Yashimoto, 2001), where the parameters of the logistic Mitscherlich growth curve were as follows: fitted parameter at t (time) and A (asymptote), estimated by R (parameter of decrease rate). Statistical tests were performed using Statistica version 10.0 (Statsoft, Inc., Tulsa, OK, USA).

RESULTS

The increase of leaf area (A) in the genotype 'Djambul2' is in Fig. 1 and dry weight (W) in Fig. 2. The highest increase in A was between the first (44 mm^2) and the third (92 mm^2) week of cultivation, when the growth of new young leaves started. In the period of root growth (4^{th}–5^{th} week), the senescent leaf tips dried out, new young leaves were formed,

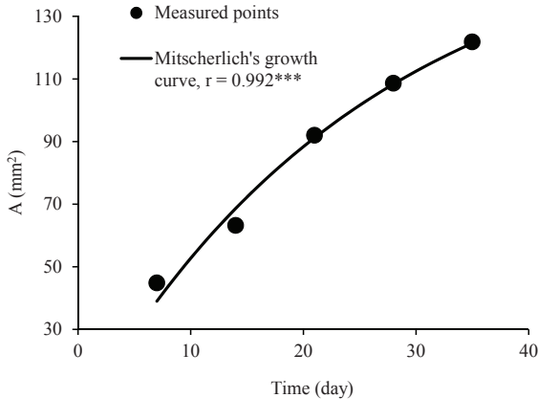

Fig. 1. Changes in leaf area (A) of genotype ´Djambul2´ in *in vitro* conditions during the period of six weeks. Measured data were fitted by Mitscherlich´s function at α = 0.001

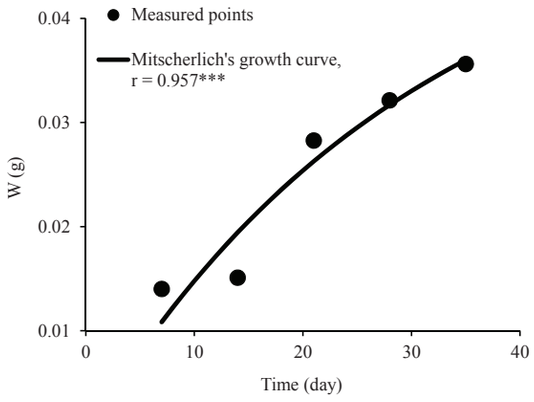

Fig. 2. Changes in dry weight of leaves (W) of genotype ´Djambul2´ cultivated in *in vitro* conditions during the period of six weeks. Measured data were fitted by Mitscherlich´s growth function at α = 0.001

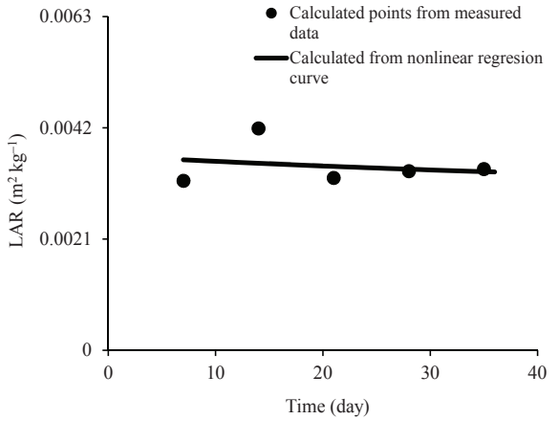

Fig. 3. Interaction effects of leaf area ratio (LAR) of genotype ´Djambul2´ cultivated in *in vitro* conditions. Calculated data were fitted by nonlinear regression curve

and the plants thickened. The dry weight (DW/leaf) increased substantially step by step. The formation of new young leaf dry matter increased rapidly between the second (0.015 g DW/leaf) and the third (0.028 g DW/leaf) week, and then the additional leaves were created in the fifth (0.035 g DW/leaf) and in the sixth (0.070 g DW/leaf) week of cultivation.

The leaf area ratio LAR (Fig. 3) of genotype 'Djambul2', estimated during the period of six weeks, remained constant throughout the culture period in *in vitro* conditions. On a percentage basis, the average increase was 8.8% for LAR. Changes in leaf area duration (LAD) of genotype 'Djambul2' in *in vitro* conditions in the period of six weeks were shown in Fig. 4. The changes were according to the nonlinear regression curve from 205 $m^2\ d^{-1}$ at the beginning measurements to 805 $m^2\ d^{-1}$ after 6 weeks.

The production rate (C) decreased throughout the whole study period (Fig. 5). This rate was expressed as an average decrease of the plant dry weight in the area of culture vessels per day. The changes in production rate were between 19 693 and 8 305 g $m^{-2}\ kg^{-1}$. The highest production rate was observed with the increasing growth of new young leaves at the third week.

In our study the relative growth rate of dry weight RGR RW (Fig. 6) decreased in relation to creation of a new assimilation leaf area (A) every week. The RGR RW dramatically decreased between the first and the second week of cultivation from 0.25 to 0.01 g $g^{-1}\ d^{-1}$, RGR RW minimally changed during the remaining

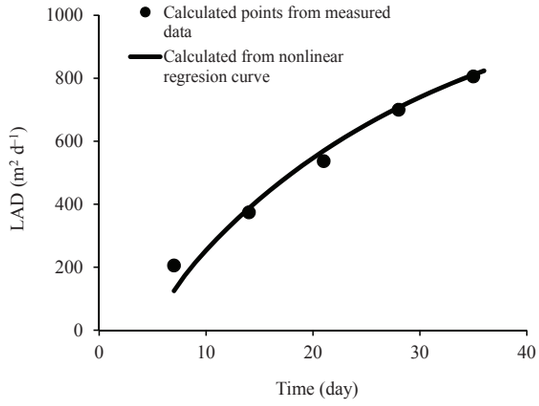

Fig. 4. Interaction effects of leaf area duration (LAD) of genotype ´Djambul2´ cultivated in *in vitro* conditions. Calculated data were fitted by nonlinear regression curve

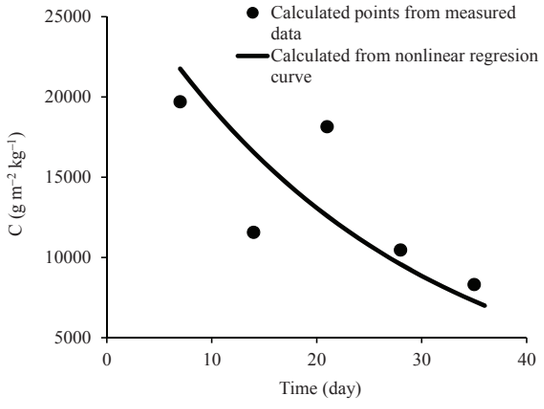

Fig. 5. Interaction effects of production rate (C) of genotype ´Djambul2´ cultivated in *in vitro* conditions. Calculated data were fitted by nonlinear regression curve

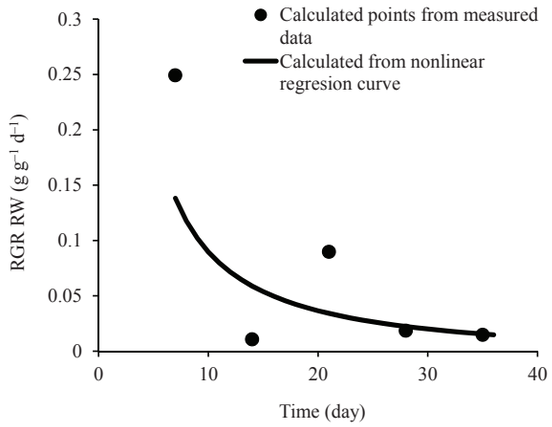

Fig. 6. Changes in relative growth rate of dry weight (RGR RW) of genotype ´Djambul2´ cultivated in *in vitro* conditions. Calculated data were fitted by nonlinear regression curve

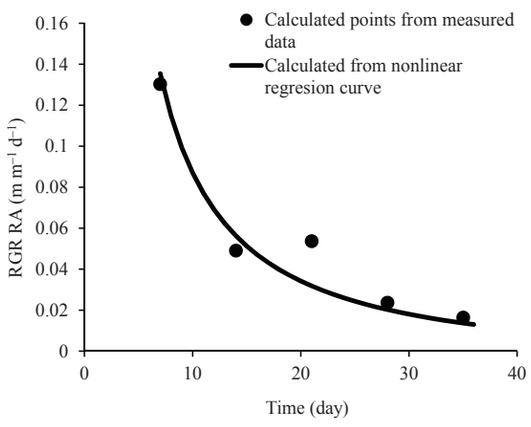

Fig. 7. Changes in relative growth rate of assimilation area (RGR RA) of genotype ´Djambul2´ cultivated in *in vitro* conditions. Calculated data were fitted by nonlinear regression curve

period of the 2nd–6th weeks. The changes in the relative growth rate of assimilation area RGR RA (Fig. 7) decreased according to the non-linear regression curve from 0.13 to 0.02 m m^{-1} d^{-1}.

Plants of *in vitro* cultures were semi-autotrophic; they used carbon from sucrose in culture medium and from CO_2. The net assimilation rate (NAR) grown throughout the studied period (Fig. 8), reflected in an increase in productivity of the assimilation organs of cultured plants. The decreasing curve of the NAR was very similar to nonlinear regression curve of the RGR RW. The NAR of the plantlet was ranging between 6.5 10^{-5} to 4.3 10^{-6} g m^{-2} d^{-1} during the period of six weeks. It was caused probably by the starting senescence of the first leaves, and the gradual dying of the older leaves tips.

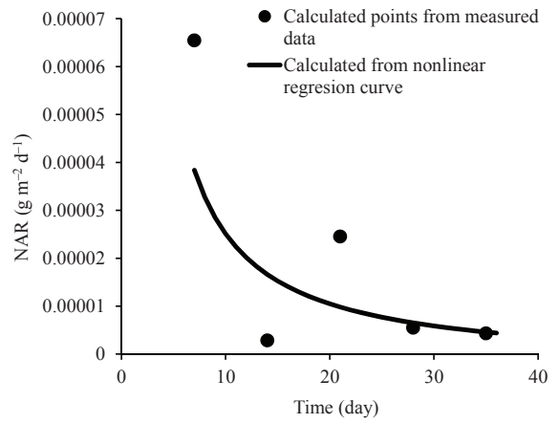

Fig. 8. Growth characteristics of garlic plants genotype ´Djambul2´ cultivated in *in vitro* conditions, from the viewpoint of net assimilation rate (NAR). Calculated data were fitted by nonlinear regression curve

DISCUSSION

There is no data in the literature about garlic growing characteristics that is why we decided to compare our observed results with the data on different plants e.g. with Kubota, Kozai (1992), who treated *Solanum tuberosum* L. in *in vitro* conditions. Their results in leaf area (A) and dry weight of leaves (W) have had similar slope as ours (Figs.1 and 2). The dry matter of young garlic leaves was lower after transplanting onto medium during the first two weeks, then it increased 3.3 times in the fifth week. The Mitscherlich´s curves represented all the points with high regression coefficient. Water content in garlic tissues (84–85%) did not change significantly throughout a culture period.

Mobini et al., (2009) compared light and heavy media (according to water holding) to the control medium at potatoes tissue cultures. They showed the increasing rate in LAD in heavy media and also it was increased in accordance to the aeration level. The LAD was 266.2 m^{-2} d^{-1} in light medium in this case. It was comparable to our results (Fig. 4) of *Allium sp*. cultivation at the beginning of experiments.

Kubota, Kozai (1992) reported for ventilation treatment of potato plant similar trend in relative growth rate of dry weight (RGR RW) as we found for nonventilated garlic (Fig. 6). They measured RGR RW 0.04 g g^{-1} d^{-1} for minimal ventilation treatment of potato plants, 0.07 g g^{-1} d^{-1} for diffusive ventilation and 0.11 g g^{-1} d^{-1} for forced ventilation of the potato plantlet during the period from 23rd to 30th days. We founded the similar RGR RW values for garlic with 3% sucrose in medium as Alvim (1960), who observed RGR RW 0.097 g g^{-1} d^{-1} for control bean plant and 0.112 g g^{-1} d^{-1} for bean plant treated in medium contained gibberellic acid and sugar. Our results for garlic were very close to the measurements with increasing salt concentration

in culture medium and subsequently reducing growth rate (Queiros et al., 2007). Balemi, Schenk (2009) calculated the RGR RW of potato genotypes in *in vitro* conditions at two harvests (after 23 and 38 days); the second harvest was significantly lower than the first, it is interesting that similar results were obtained for garlic.

The study by Alvim (1960) reported the NAR of bean 0.035 g dm^{-2} d^{-1} for control plant and 0.042 g dm^{-2} d^{-1} for plant treated in medium contained gibberellic acid and sugar. He reported that sugars also seemed to have a positive effect on increasing NAR and RGR RW what is in agreement with cultivation of garlic in culture medium with 30 g l^{-1} sucrose (Fig. 8). Our results confirmed the limitation of nutrition resources in the culture media.

CONCLUSION

The results describing the growth characteristics of garlic plants genotype 'Djambul2' in *in vitro* conditions were presented. This paper summarized the growth analysis of garlic cultivation in *in vitro* conditions: the leaf area (A), dry weight of leaves (W) and the leaf area duration (LAD) increase significantly. The leaf area ratio (LAR) was constant throughout the culture period. The production rate (C) was a decreased trend. The relative growth rate of dry weight (RGR RW), the relative growth rate of assimilation area (RGR RA), and the net assimilation rate (NAR) approached to zero in *in vitro* conditions after six weeks. These results characterized the growth of garlic plant in *in vitro* conditions and can be a base for further work.

REFERENCES

ALVIM, P.D.T.: Net assimilation rate and growth behavior of beans as affected by gibberellic acid urea and sugar sprays. Plant Physiology, *35*, 1960: 285–288.

AYABE, M. – SUMI, S.: A novel and efficient tissue culture method - 'stem-disc dome culture' - for producing virus-free garlic (*Allium sativum* L.). Plant Cell Reports, *20*, 2001: 503–507.

BALEMI, T. – SCHENK, M.K.: Genotypic variation of potato for phosphorus efficiency and quantification of phosphorus uptake with respect to root characteristics. Journal Plant Nutrition Soil Science, *172*, 2009: 669–677.

ENGELMANN, F.: Biotechnologies and conservation of phytogenetic resources. Cahiers Agriculture, *18*, 2009: 481–485.

EVANS, G.C.: The Quantitative Analysis of Plant Growth. Berkeley and Los Angeles, USA, University of California Press, 1972.

HUNT, R. – CAUSTON, D.R. – SHIPLEY, B. – ASKEW, A.P.: A modern tool for classical plant growth analysis. Annals of Botany, *90*, 2002: 485–488.

KAMENETSKY, R.: Life-cycle, flower initiation, and propagation of the desert geophyte *Allium rothii*. International Journal of Plant Sciences, *155*, 1994: 597–605.

KUBOTA, C. – KOZAI, T.: Growth and net photosynthetic rate of *Solanum tuberosum in vitro* under forced and natural ventilation. Horticultural Science, *27*, 1992: 1312–1314.

LAMBERS, H. – CHAPIN, F.S. – PONS, T.L.: Plant Physiological Ecology. Germany, Springer, 2008.

LE GUEN-LE SAOS, F. – HOURMANT, A. – ESNAULT, F. – CHAUVIN, J.E.: *In vitro* bulb development in shallot (*Allium cepa* L. *aggregatum* group): Effects of anti-gibberellins, sucrose and light. Annals of Botany, *89*, 2002: 419–425.

MITSCHERLICH, E.A.: The second approach of the action of the law growth factors. Zeitschrift für Pflanzenernährung, *12*, 1928: 273–282. (in German)

MOBINI, S.H. – ISMAIL, M.R. – AROUIEE, H.: Influence of ventilation and media on potato (*Solanum tuberosum* L.) tuberization and its growth characteristics. African Journal of Biotechnology, *8*, 2009: 2232–2241.

MURASHIGE, T. – SKOOG, F.: A revised medium for rapid growth and bio assays with tobacco tissue cultures. Plant Physiology, *15*, 1962: 473–483.

QUEIROS, F. – FIDALGO, F. – SANTOS, I. – SALEMA, R.: *In vitro* selection of salt tolerant cell lines in *Solanum tuberosum* L. Plant Biology, *51*, 2007: 728–734.

WU, Z. – CHEN, L.J. – LONG, Y.J.: Analysis of ultrastructure and reactive oxygen species of hyperhydric garlic (*Allium sativum* L.) shoots. *In vitro* Cellular and Developmental of Biology-Plant, *45*, 2009: 483–490.

YOSHIMOTO, A.: Application of the logistic, Gompertz, and Richards growth functions to Gentan probability analysis. Journal of Forest Research, *6*, 2001: 256–272.

Received for publication on August 15, 2012
Accepted for publication on September 6, 2012

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Růstové charakteristiky česneku v *in vitro* podmínkách

Scientia Agric. Bohem., *43*, 2012, 85–90

V této práci byly studovány základní charakteristiky růstu rostlin (*Allium sativum* L.) genotype **'**Djambul2**'** v *in vitro* podmínkách. V týdenním intervalu byly popisovány růstové charakteristiky Mitscherlichovou funkcí. Asimilační plocha listu (A), průměrná sušina listu (W) a integrální listová plocha (LAD) vykazovaly nárůst hodnot. Změny hodnot poměrné olistěnosti (LAR) narůstaly do 8.8 % v průběhu experimentu. Snižující trend byl prokázán u přírůstku hmotnosti sušiny (C) o 42 %, relativní rychlosti růstu sušiny (RGR RW) 5.8 % a asimilační plochy (RGR RA) 12.6 %, a čistého výkonu asimilace (NAR) 6.6 % v průběhu šesti týdenní kultivační periody. Tato zjištění podporují hypotézu o omezení zdrojů výživy v kultivačním médiu.

Allium sp.; tkáňové kultury; poměrná olistěnost; přírůstek hmotnosti sušiny; čistý výkon asimilace

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