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Jasmonic Acid Stimulates Shoot and Bulb Formation of Garlic In Vitro

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Abstract. Although much information is available concerning the involvement of jasmonates in the regulation of plant development, few reports are devoted to their effects in tissue culture. In the present study, the influence of jasmonic acid (JA) on shoot and bulb formation in tissue culture of garlic (Allium sativum cv. Ptuj) was studied. Isolated basal plates were placed onto Gamborg's B5 medium. JA significantly enhanced the shoot and bulb development in concentrations from 1–10 μM. When the combination of 10 μM JA and 5 μM 2-iP was used in the initiation media, the average number of shoots was 30 after 6 weeks of culture. The bulbs formed on approximately 50% of shoots. Results described in this article show that JA might play an important role in the formation of storage organs in plants, in this case on garlic bulbs.

Jasmonic acid (JA) and related compounds are known to be distributed widely among higher plants (Meyer et al. 1984) and play important roles in the regulation of plant development (reviewed by Koda 1992, Sembdner et al. 1989).

Apart from the promotion of senescence (reviewed by Parthier 1990, Staswick 1992), the induced accumulation of the jasmonate-induced polypeptides (JIPs) was intensely investigated (Anderson 1988, Herrmann et al. 1989, Weidhase et al. 1987b). It was found that jasmonates are potent inducers of vegetative storage protein gene expression (Staswick 1990) and defense proteins–proteinase inhibitors (Farmer and Ryan 1990, 1992). Many researchers proposed that jasmonates could play a role of second messenger in plants (Anderson 1989, Farmer and Ryan 1990, 1992, Gundlach et al. 1992).

Our previous investigations have shown that jasmonic acid influences different physiological processes in plants in vitro. JA altered the potato protoplast membrane fluidity (Vilhar et al. 1991), enhanced cell division and microcalli development in protoplast cultures (Ravnikar et al. 1992), and stimulated the development of shoots on isolated potato meristems (Ravnikar and Gogala 1990). In potato and grapevine stem cultures, concentrations of up to 1 μM ± JA increased the shoot length and influenced adventitious rhizogenesis by stimulation of lateral root formation and enlargement of root hairs (Blažina et al. 1991, Ravnikar et al. 1990, 1992).

Jasmonic acid and some other jasmonates were found to be strong endogenous tuber-inducing substances in the dicot potato plants and in the monocot yam plants (reviewed by Koda 1992). Koda (1992) suggests that jasmonates control bulb and tuber formation.

Garlic is only propagated vegetatively (Novak 1990). In vitro methods are suitable for overcoming the problems connected with conventional propagation by cloves: a low coefficient of multiplication and uncontrolled spread of viral diseases. Descriptions of the garlic viruses and their elimination by thermotherapy and meristem culture have been described frequently (Ayuso and Pena-Iglesias 1981, Conci and Nome 1991, Van Dijk et al. 1991, Walkey et al. 1987), while there are only a few reports dealing with successful micropropagation of garlic. Flower heads and basal stem plates were used as explants (Bhojwani 1980, Novak 1990). Usually, the regeneration of shoots and the transfer of rooted plantlets was achieved; however, microbulbs formed in vitro only by chance.

In view of the cited information, we decided to observe the influence of JA on shoot and bulb formation in tissue cultures of garlic.

Materials and Methods

Plant material

Bulbs of garlic (Allium sativum L. cv. Ptuj) were maintained at 4°C in the dark. Cloves were dipped in 70% ethanol, surface-sterilized in 2.4% Na-hypochlorite, containing a few drops of

Tween 80 for 15 min and rinsed three times in sterile bidistilled water. The basal plates were taken as explants.

Culture Media and Conditions

Gamborg's B5 medium (Gamborg et al. 1968) was used for all experiments. The media were supplemented with 0.01–10 μM (±) jasmonic acid (SA Firmenich, Geneve, Switzerland). In experiments 2 and 3, also 2-iP (N^6(2-Isopentenyl)adenine, Sigma D 8532) in the concentration of 5 μM was used. This concentration showed best bud induction in the preliminary experiments. pH was adjusted to 5.7 before autoclaving. Cultures were transferred to fresh media every 3 weeks. They were maintained at 23 ± 2°C, 46–55 $\mu Em^{-2}s^{-1}$ illumination, and 16 h light/8 h dark photoperiod.

Experimental procedures

Three different experimental approaches were used to investigate the effects of JA in garlic tissue culture.

Experiment 1. The stimulation of shoot and bulb development was observed under the influence of JA. JA was added to the media at concentrations of 0, 0.01, 0.1, 1, and 10 μM. Every 3 weeks cultures were transferred to fresh media with the same concentrations of JA.

Experiment 2. The influence of JA on bulb induction was investigated using previously developed shoots. Five micro mols of 2-iP was added to the medium for shoot induction. On average, three additional shoots developed on the isolated basal plates after 3 weeks. Cultures were transferred onto the media without 2-iP, but were supplemented with 0, 0.01, 0.1, 1, and 10 μM JA and kept on the same media until the end of the experiments.

Experiment 3. Shoot initiation was observed under the influence of JA and 2-iP used simultaneously. Shoot induction media were supplemented with 5 μM 2-iP and 0, 1, 2.5, 5, 7.5, or 10 μM JA. After 3 weeks, cultures were transferred to the media with the same concentrations of JA but without 2-iP.

Every 3 weeks the number and length of shoots (and later the number and the diameter of bulbs) were measured. In all experiments, 10–15 explants were used for each treatment and all were repeated at least twice.

Statistics

The student *t*-test was used for evaluating the levels of significant differences between the control media without JA and those supplemented with JA. Symbols used in the figures are: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; bars mean 2× standard error (SE).

Results

After 2–3 weeks, newly formed shoots started to grow on the apical part of the isolated basal plates if JA or 2-iP were added to the medium. More shoots developed in the next 3 weeks. Bulbs were formed between weeks 6 and 9 of culture, depending on the experimental approach.

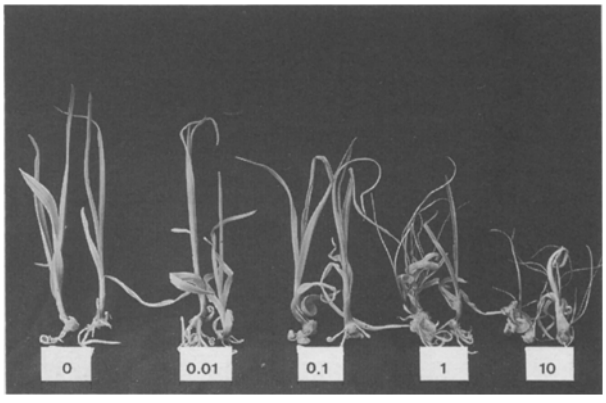

Fig. 1. Explants grown on media with 0, 0.01, 0.1, 1, and 10 μM JA after 3 weeks of culture.

In experiment 1 (see Materials and Methods), the stimulation of shoot and bulb development was observed under the influence of JA. After 3 weeks, only leaves of terminal buds and adventitious roots developed on the isolated basal plates placed on the control medium and that with 0.01 μM of JA. At higher concentrations of JA, additional shoot development was stimulated (Fig. 1). However, the number of developed shoots was low even after 6 weeks of culture (Fig. 2). We also observed that with increasing JA concentration, the inhibition of growth of the terminal bud leaves and roots became more marked. After 6 weeks, the formation of bulbs on the control medium and that with 0.01 μM JA occurred only rarely on the basal part of the terminal shoot. At 1 and 10 μM JA, a significantly higher number of bulbs developed (Fig. 2).

In experiment 2, axillary shoots were first initiated on the media with 2-iP, and after 3 weeks of culture the explants were placed onto the media supplemented with JA. The stimulation of bulb formation was most evident after an additional 3 weeks on 10 μM JA. Nearly all additionally developed shoots formed bulbs whose diameter increased on the same concentration (Fig. 3).

In experiment 3, the shoot induction medium contained both 2-iP and JA; the number of additional shoots increased with increasing concentration of JA; the shoot length simultaneously decreased (Fig. 4). After 3 weeks, cultures were transferred onto media with only JA. During the first subculture, the number of shoots almost doubled (Table 1). Bulb formation was delayed 3–6 weeks as compared to experiments 1 and 2, and started during the third subculture. After 12 weeks, a large number of bulbs developed (Fig. 5, Table 1). The

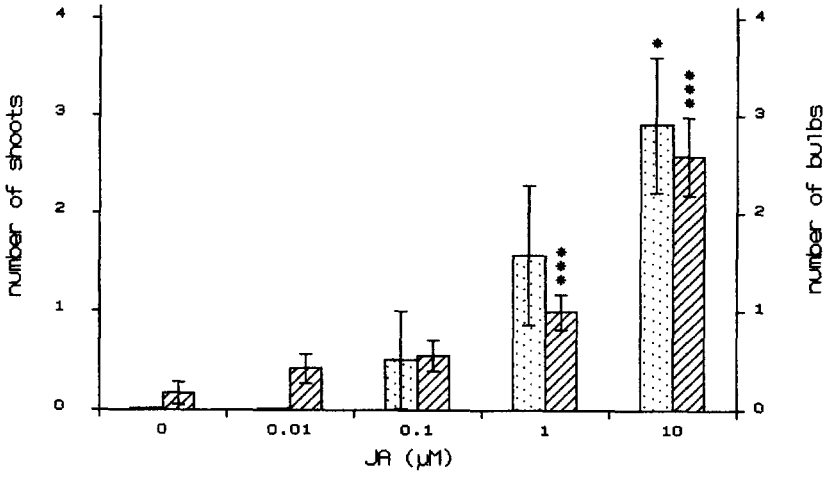

Fig. 2. The effect of JA on the number of shoots (dotted columns) and bulbs (dashed columns) after 6 weeks in culture.

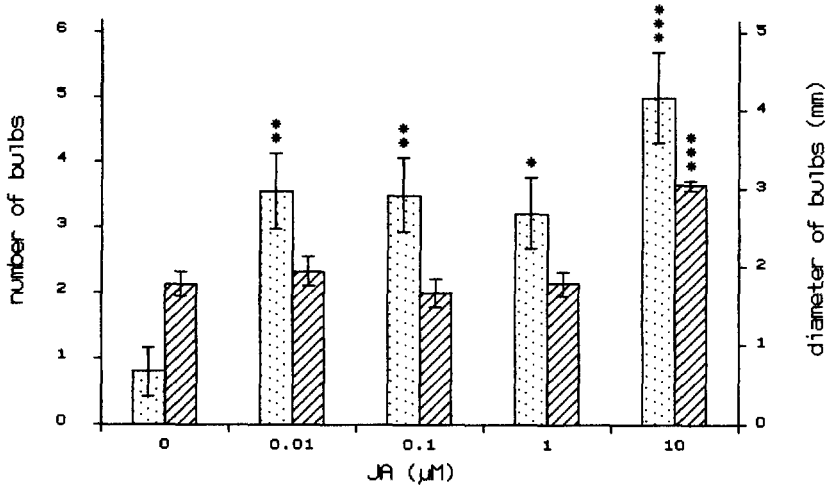

Fig. 3. The effect of JA on the number (dotted columns) and diameter (dashed columns) of bulbs formed on the shoots initiated by 5 μM 2-iP after 6 weeks in culture.

significant increase of bulbs corresponded with the increased concentration of JA in the medium. Additionally, the bulbs formed on approximately 50% of shoots on the media with JA, while only 11.6% of the shoots developed bulbs on the control medium (Table 1). After 12 weeks, the bulbs were separated from the starting explant, and after 3–4 months of culture they started to germinate.

Discussion

Our results show that the introduction of JA in tissue culture of garlic has beneficial effects on shoot and bulb development. As mentioned before, only a few reports deal with successful garlic micropropagation. As reported by Bhojwani (1980), the average rate of shoot formation per explant was 8 after 6 weeks of culture. In our experiments, the number of developed shoots was very high when the combination of JA and 2-iP was used in the initiation media; the average number of shoots was 18 after 3 weeks and 30 after 6 weeks. The synergistic effect of JA and cytokinins was previously observed in potato protoplast culture (Ravnikar et al. 1992). In those experiments, JA was added to the medium which included both cytokinin and auxin. Stimulation of cell division and microcalli development was observed, accompanied by the enlargement of cells which developed from protoplasts on the media with JA (unpublished results). JA also stimulated the development of shoots on isolated potato meristems (Ravnikar and Gogala 1990).

On the other hand, there are also many reports which indicate that jasmonates and cytokinins have antagonistic modes of action (Ueda and Kato 1982, Weidhase et al. 1987a). It appears that this depends on the plant species, the target tissue, and its sensitivity.

The stimulatory effect of JA on bulb formation

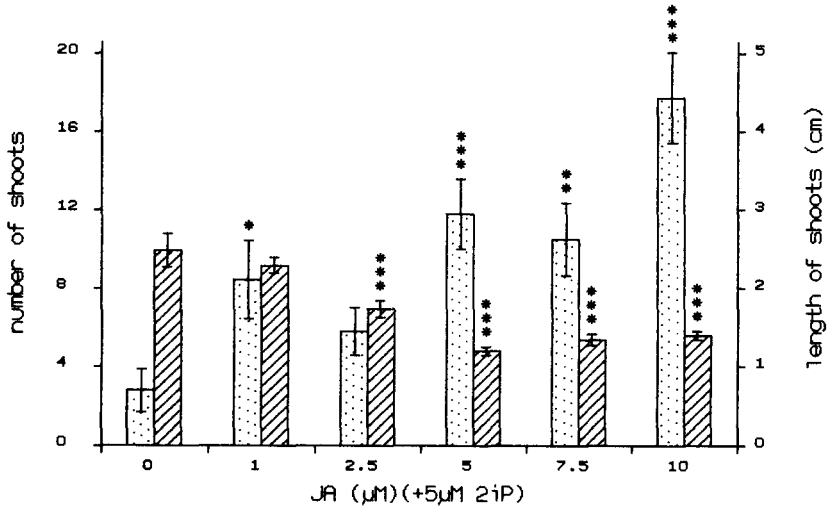

Fig. 4. The effect of JA in different concentrations and 5 μM 2-iP on the number (dotted columns) and length (dashed columns) of shoots after 3 weeks in culture.

Table 1. Development of shoots and bulbs on media with 5 μM 2-iP and increasing concentrations of JA.

	JA (μM)					
	0	1	2.5	5	7.5	10
No. of starting explants	15	13	15	16	14	14
Total no. of shoots after 3 weeks	42	110	87	177	147	248
Total no. of shoots after 6 weeks	78	125	149	278	268	421
Total no. of bulbs after 12 weeks	9	44	74	131	171	235
% of shoots developing bulbs	11.6	35.2	49.7	47.1	63.8	55.8

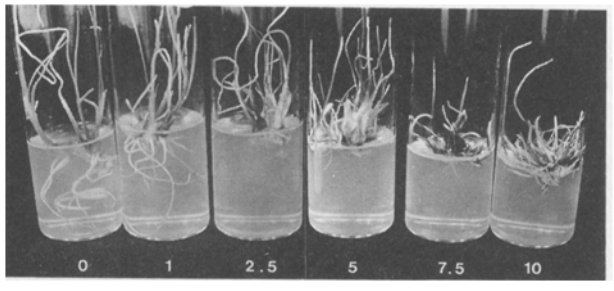

Fig. 5. Bulb formation after 3 months of culture on media with 0, 1, 2.5, 5, 7.5, and 10 μM JA on explants initiated by the same concentrations of JA and 5 μM 2-iP.

was proposed as jasmonates show strong tuber-inducing activity in potato and some other plants (Koda 1992). In particular, jasmonate-dependent potato tuber formation was intensively investigated (Koda et al. 1991, Van den Berg and Ewing 1991).

In the formation of potato tubers, lateral expansion of cells in the tip of the stolon occurs. Our previous observations show that exogenous jasmonic acid in potato node cultures also causes the expansion—in that case, the expansion of root hairs (Ravnikar et al. 1990).

The formation of onion bulbs is also brought about by lateral expansion of leaf-sheath cells. It is accompanied by the disruption of cortical microtubules in the cells (Mita and Shibaoka 1983). Abe and coworkers (1990) reported that, in tobacco cell culture, jasmonates induced the disruption of cortical microtubules. Therefore, they postulated that jasmonates might be involved in the formation of storage organs such as potato tubers and onion bulbs. The results described in this article suggest the involvement of JA in the formation of storage organs in plants—in this case, on garlic bulbs.

The observed enhancement of microbulb formation by JA can be regarded as a possible improvement of garlic micropropagation by way of bulb formation. Even if the average number of shoots and bulbs was highest on the medium with 10 μM of JA, we suggest the application of 5 and 7.5 μM JA, as the bulbs are larger at these concentrations and, as such, are easier to manipulate further.

Considering the many interesting effects of JA observed in different tissue culture systems, the jasmonates should be included in studies of in vitro procedures such as protoplast regeneration, and investigations of applied micropropagation (e.g., ad-

ventitious root development, axillary bud development, etc.).

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