J Plant Growth Regul (1990) 9:233–236

Journal of
Plant
Growth
Regulation

Regulation of Potato Meristem Development by Jasmonic Acid In Vitro

Maja Ravnikar[1] and Nada Gogala[2]

[1]Institute of Biology, University of Ljubljana, Aškerčeva 12, 61 000 Ljubljana, Yugoslavia; and [2]Department of Biology, Biotechnical Faculty, University of Ljubljana, Aškerčeva 12, 61 000 Ljubljana, Yugoslavia

Received September 13, 1989; accepted March 14, 1990

Abstract. The influence of jasmonic acid (JA) on differentiation of meristems of the potato, *Solanum tuberosum cv. Vesna,* was investigated in vitro. Meristems were grown on Murashige and Skoog (MS) medium supplemented with indole-3-acetic acid (IAA) (10 μM), kinetin (10 μM), gibberellic acid (3 μM), as modified by Bang. Addition of JA in concentrations of 0.5–10 μM increased the number of meristems that developed into buds, particularly in meristems isolated from shoots grown from tubers in the dark. JA had no noticeable effect on meristems from germs grown in light. All added concentrations of JA retarded callus and root formation. The inhibitory effect on rhizogenesis disappeared immediately after transfer of the developed buds to medium without JA.

Jasmonic acid (JA) and its methyl ester (JA-Me) have been found to be ubiquitously distributed in plants (Dathe et al. 1981, Meyer et al. 1984, Miersch et al. 1986, Yamane et al. 1981). Jasmonates have also been found to possess different physiological activities when exogenously applied to plants (see Sembdner and Gross 1986 for references); in particular, the senescent effect of JA has been studied extensively.

There are only a few reports on the in vitro effects of jasmonates. JA inhibited cytokinin-induced callus growth of *Glycine max* (Ueda and Kato 1982). Polashock and Mott (1987) reported on the effects of JA on embryogenic cultures of Norway spruce. JA stimulated the longitudinal growth of shoots of grapevine and potato stem cuttings (Ravnikar et al. 1990) and lateral root development on potato cuttings (Ravnikar and Gogala 1988).

Knöfel and coworkers (1984) found higher concentrations of JA in shoot and root apices than in cotyledons and other root segments; similar results were obtained with respect to the root apex of *Vicia faba* and *Pisum sativum* (Sembdner and Klose 1985). Therefore, we presumed a role for JA in meristem development.

This work presents the influence of JA on potato meristem development in vitro.

Materials and Methods

Potato tubers *Solanum tuberosum* L. *cv.* Vesna were stored in the dark at 4°C. They were germinated as one-eyed parts in soil, in light at 25°C [light-grown germs (LGG)], or in the dark at the same temperature [dark-grown germs (DGG)]. Three- to 4-week-old plants were sterilized for 10 s with 70% ethanol followed by 10 min with 5% Ca-hypochlorite and then rinsed three to four times with sterile double-distilled water.

The meristem apices (0.3–1 mm in length with two to four leaf primordia) were dissected and placed on Murashige and Skoog (MS) medium (1962) modified by Bhojwani and Razdan (1983) as follows: $FeSO_4 \cdot 7H_2O$ and FeEDTA · $2H_2O$ were replaced with NaFeEDTA (136 μM) and $ZnSO_4 \cdot 7H_2O$ was used in a concentration of 36.85 μM. DifcoBacto agar at 0.6–0.7% and 3% sucrose were added. This basal medium was supplemented with adenine (590 μM), indole-3-acetic acid (IAA), (10 μM), kinetin (10 μM), and gibberellic acid (3 μM) (Bang 1979) to obtain the meristem growth induction medium (control medium), chosen on the base of preliminary results. (±)JA (Firmenich SA, Geneva, Switzerland) was added to the control medium in concentrations of 0.5–25 μM. Media were adjusted to pH 5.7–5.8 before autoclaving.

Cultures were kept at 25 ± 2°C, with a photoperiod of 16 h at 5,5–12,3Wm^{-2} (Sylvania Gro Lux F40T12 and fluorescent LV 20 lights). Cultures were transferred to fresh medium every 4 weeks. Developed plants were multiplied with segmentation on basal medium and transferred to a greenhouse.

Student's *t* test and a chi-squared contingency table (2 × 2) with Fisher's correction were used to calculate the levels of significant differences between the control medium and media supplemented with JA (De Fossard 1976). The following symbols are used: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, where p means the level of significant difference between growth on control medium and media with JA and is 2× the standard error (SE). Fifteen to 20 meristems were used for each concentration of JA and the control medium, and all experiments were repeated three or more times.

Fig. 1. Multiple budding on medium with JA, 4 weeks after meristem isolation.

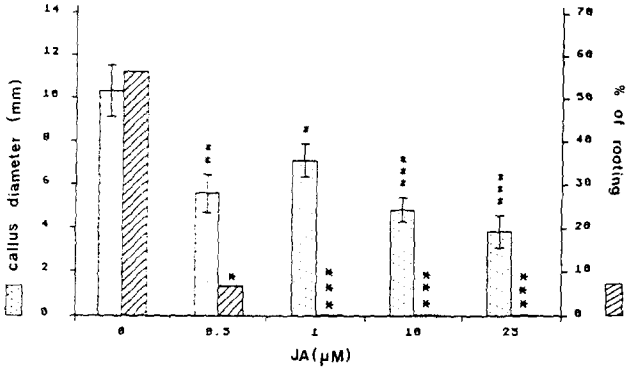

Fig. 2. The inhibition of callus and root development by JA, 4 weeks after meristem isolation.

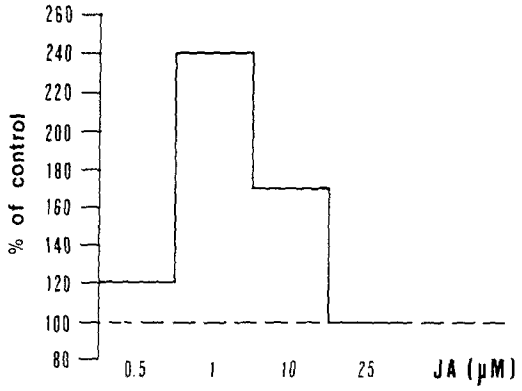

Fig. 3. The influence of JA on the number of meristems (DGG) developing buds after 4 weeks in culture.

Results

Development of Plants from Meristems on Control Medium

MS medium as modified by Bang enabled 97% meristem survival. Shoots appeared 2–3 weeks after the meristems were dissected and placed on medium. This was independent of their LGG or DGG origin. About 2–5-cm tall, rooted plantlets were obtained in 4–6 weeks. On isolated meristems, callus appeared and adventitious buds developed beside axillary buds. The number of buds formed on individual meristems was relatively high, averaging five. In 20% of the meristems, up to 40 buds appeared on a single meristem (Fig. 1).

Influence of JA on Callus Formation

The diameter of the base of the callus was measured. JA significantly inhibited callus formation in all applied concentrations (Fig. 2). These results demonstrate that the mode of reaction is again independent of the origin (LGG or DGG).

Influence of JA on Bud Formation

The number of buds formed on a single meristem was comparable to the control when JA in concentrations of 0.5–3 μM was added to the medium. Concentrations higher than 10 μM retarded development and growth.

The number of meristems forming buds under the influence of JA was dependent on their origin (LGG or DGG). In the experiments with LGG, the influence of JA on bud formation was not noticeable, but when DGG was used as the starting material, there was significant stimulation at concentrations of 0.5–10 μM JA (Fig. 3).

Influence of JA on Root Development

The number of buds forming roots was measured.

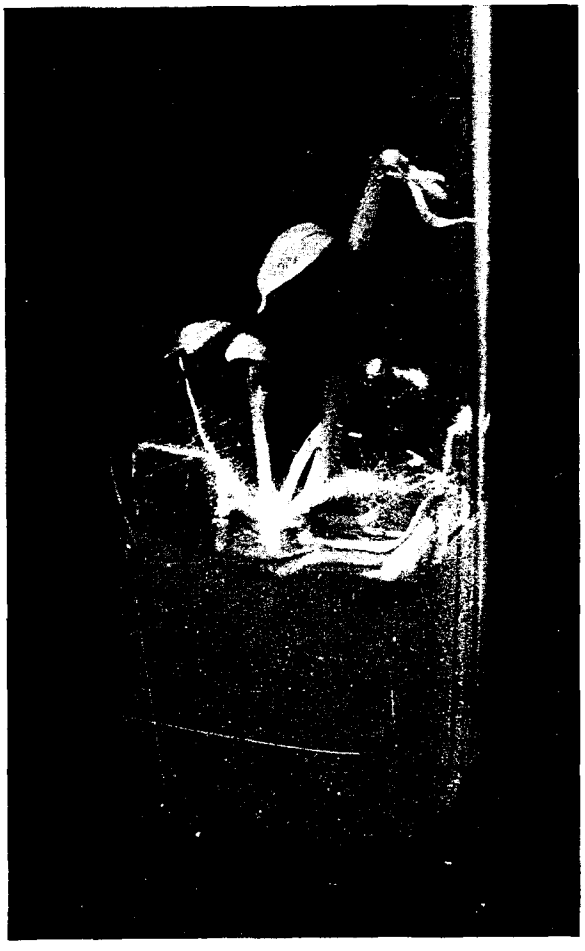

Fig. 4. Buds with developed roots after 2 weeks on basal medium.

In comparision with the control medium, JA significantly inhibited root formation (Fig. 2). The inhibition was nontoxic, since all regenerated buds formed roots as soon as they were transferred to the basal medium (Fig. 4). The length of time (2, 4, or 6 weeks) of shoot growth on JA-supplemented media had no effect on normal root formation after transfer to the basal medium. Developed plants adapted to greenhouse conditions without any problems.

Discussion

Experimental data indicate a physiological role of JA in meristem development. JA can influence organogenesis by inhibition of callus and root formation and enhancing bud formation. A similar callus–inhibition effect was observed by Ueda and Kato (1982) on cytokinin-induced callus growth.

In our experiments, JA prevented in vitro root formation. This effect is contrary to rhizogenesis in potato stem cuttings (Ravnikar and Gogala 1988), where addition of JA in the medium stimulated lateral root formation and resulted in a well-developed root system. Similar results were obtained by Zimmerman and Vick (1983), who showed that JA stimulated development of adventitious roots in *Vigna radiata*.

In DGG, JA increased the number of meristems that developed buds. Differences in the source material are probably the cause of differences in concentrations of endogenous plant growth regulators (Pharis and Reid 1985). Meyer and coworkers (1984) found differences in JA concentrations between dark- and light-grown potato germs. However, the data are not directly comparable to our experiments, which were performed with different cultivars.

Meristem differentiation, among others, is the result of combined influences of endogenous plant growth regulators and applied kinetin, IAA, gibberellic acid, and JA. However, we observed that plantlets were more vigorous on medium with JA. This observation found its first application in the production of virus-free potato plants via meristem culture (Ravnikar and Gogala 1989).

Acknowledgments. We wish to thank Drs. Sembdner and Miersch (Institute for Biochemistry Haalle/Saale, DDR) for helpful discussions and the gift of JA and the Institute for Potato Virus Diseases and Physiology (Kranj, Yugoslavia) for the starting plant material. We are also grateful to M.Sc. Jana Žel for constructive comments and Nada Hojnik and Vanda Golobinek for technical assistance.

References

Bang U (1979) Totally infected potato varieties cured from virus diseases by meristem culture. Sveriges Lanttbruksuniversitet Vaxtskyddsrapporter Jordbruk 10:1–26

Bhojwani SS, Razdan MK (1983) Plant tissue culture, theory and practice. In: Development in crop science (5). Elsevier, Amsterdam, Oxford, New York, Tokyo, pp 25–41

Dathe W, Rönsch H, Preiss A, Schade W, Sembdner G, Schreiber K (1981) Endogenous plant hormones of the broad bean, *Vicia faba* L. (−)-Jasmonic acid, a plant growth inhibitor in pericarp. Planta 155:530–535

De Fossard RA (1976) Tissue culture for plant propagators. University of New England Printery, Australia, pp 95–131

Knöfel HD, Brückner C, Kramell R, Sembdner G, Schreiber K (1984) A radioimmunoassay for jasmonic acid. Biochem Physiol Pflanzen 179:317–325

Meyer A, Miersch O, Büttner C, Dathe W, Sembdner G (1984) Occurrence of the plant growth regulator jasmonic acid in plants. J Plant Growth Regul 3:1–8

Miersch O, Meyer A, Vorkefeld S, Sembdner G (1986) Occurrence of (+)-7-iso-jasmonic acid in *Vicia faba* L. and its biological activity. J Plant Growth Regul 5:91–100

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Pharis PP, Reid DM (1985) Hormonal regulation of development III. Role of environmental factors. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo

Polashock JJ, Mott RL (1987) The effects of the plant growth regulators abscisic acid, gibberellic acid-4-plus-7, and jasmonic acid on embryogenic cultures of norway spruce. Plant Physiol 83(suppl 4):77

Ravnikar M, Gogala N (1988) The regulation of potato meristems development by jasmonic acid in vitro. 6th FESPP Congress, September

Ravnikar M, Gogala N (1989) The influence of jasmonic acid on development of virus-free potato plants in vitro. Biol Vest 37:79–88

Ravnikar M, Gogala N, Benedičič D, Rode J (1990) Regulation of organogenesis with jasmonic acid. Acta Hortic (in press)

Sembdner G, Klose C (1985) (±)Jasmonic acid—a new phytohormone? Biol Rdsch 23:29–40

Sembdner G, Gross D (1986) Plant growth substances of plant and microbial origin. In: Bopp M (ed) Plant growth substances 1985. Springer-Verlag, Berlin, Heidelberg, New York, pp 139–147

Ueda J, Kato J (1982) Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiol Plant 54:249–252

Zimmermann DC, Vick BA (1983) Stimulation of adventitious rooting in mung bean *Vigna radiata* seedlings by jasmonic acid and 12-oxo-phytodienoic acid. Plant Physiol (suppl 72):108

Yamane H, Takagi H, Abe H, Yokota T, Takahaski N (1981) Identification of jasmonic acid in three species of higher plants and its biological activities. Plant Cell Physiol 22:689–697