

INHIBITORY EFFECTS OF METHYL JASMONATE ON SEED GERMINATION IN MAIZE (*ZEA MAYS*): EFFECT ON α -AMYLASE ACTIVITY AND ETHYLENE PRODUCTION

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Summary. Methyl jasmonate (MeJA) inhibited germination and root elongation of maize seeds. There was a reciprocal relationship between MeJA concentrations applied and seed germination and radicle growth. Determination of α -amylase activity in crude extracts and zymogram analysis showed that both activity and content of the enzyme decreased in the presence of MeJA. In addition, a reduction in ethylene production which is necessary for seed germination was also observed upon MeJA treatment. Consequently, our data showed that decreased α -amylase activity and/or concentration as well as reduced ethylene production can play significant roles in the physiological and biochemical processes underlying inhibited seed germination and root elongation in maize under conditions of increased MeJA concentrations.

Key words: methyl jasmonate, ethylene, α -amylase, maize.

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Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid, ABA - abscisic acid, DNS - 3,5-dinitrosalicylic acid, GA - gibberelic acid, Mes - (2-morpholino)-ethansulphonic acid, MeJA - methyl jasmonate, SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis.

INTRODUCTION

Synthetic jasmonates exert different physiological and biochemical effects in plants including inhibition of stem and root growth (Staswick et al., 1992), induction of leaf senescence (Yeh et al., 1995; Ananieva et al., 2004), inhibition of chlorophyll formation and carotenoid synthesis (Saniewski and Czapski, 1983) and reduction in photosynthetic (Rossato et al., 2002) and respiratory activities (Popova et al., 1988). The involvement of jasmonic acid and its methyl ester in seed germination and plant aging is one of the long recognized effects of jasmonates. There are many reports showing that MeJA inhibits germination of seeds in angiosperms, such as *Amarantus caudatus* (Kepcznski and Bialecka, 1994; Bialecka and Kepcznski, 2003b), cocklebur (Nojavan and Ishizawa, 1998), sunflower (Corbineau et al., 1988), tobacco (Preston et al., 2002) and rapeseed and flax (Wilen et al., 1994). The decrease in root growth is one of the consequences of germination inhibition (Corbineau et al., 1988; Staswick et al., 1992). Inhibition of seed germination and root elongation increases with increasing concentrations of MeJA. One of the reasons for this inhibition may be related to ethylene production which is suspected to be a required factor in seed formation and germination (Nojavan-Asghari and Ishizawa, 1998). The role of ethylene in germination remains controversial. Some authors believe that gas production is a consequence of the germination process while others consider ethylene production as a requirement for germination. Furthermore, among seeds that require ethylene, some of them are extremely sensitive to the gas whereas others require relatively high levels to trigger germination (Matilla, 2000). Therefore, we assumed that this inhibition is related to endogenous release of ethylene and it has been reported that application of exogenous MeJA causes the inhibition of germination by

reducing ethylene production (Nojavan-Asghari and Ishizava, 1998; Kepczynski, et al., 1999). This inhibition can be prevented by ethephon (an ethylene-releasing compound) (Kepczynski and Bialecka, 1994; Yildiz and Yilmaz, 2002), exogenous ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene biosynthesis (Nojavan-Asghari and Ishizava, 1998; Kepczynski et al., 1999).

During seed germination an increase in amylolytic activity in the endosperm is observed which is due mainly to secretion of α -amylases by the adjoining aleurone layer and scutellum. It has been reported that MeJA could reduce α -amylase activity during seed germination (Bialeca and Kepczynski, 2003a), but it has not been specified whether this reduced activity can be due to a decrease of the specific activity or to inhibited enzyme production. In the present study, we examined the effects of exogenous MeJA on both seed germination and root growth of maize. To elucidate these effects α -amylase activity and ethylene production were determined.

MATERIALS AND METHODS

Seed germination and radicle growth

Maize (*Zea mays* L. genotype single cross 704) seeds were obtained from Iranian agricultural research center (2004). The seeds were washed with distilled water and sterilized with 5% (w/v) sodium hypochlorite solution for 10 min. After that seeds were incubated in 15 ml 5% ethanol (v/v) (control) or different concentrations of MeJA (50, 100, 200 and 500 μ M) in 15 cm Petri dishes at 25 °C in darkness. All different concentrations of MeJA were dissolved in 5% ethanol (v/v) as a solvent. After 4 days the percentage of seed germination was determined. A seed was regarded as germinated when its radicle had protruded about 1mm from the seed coat. The lengths of radicles were also measured.

Determination of α -amylase activity

For α -amylase assay and electrophoretic analysis, experiments were

carried out with seeds treated as above. After 2 days, soaked seeds were dried with filter paper and then 3 g of seeds from each treatment were weighed and ground in 12 ml of grinding media consisting of 50 mM MES, pH 6, 5 mM CaCl₂ and glycerol 5% (v/v) in ice-cold condition. The homogenate was filtered through Miracloth and centrifuged at 15,000 g for 15 min to clarity and desalted through Sephadex G-25 gel (P10 column). The activity of α -amylase of crude enzyme was determined at room temperature by the dinitrosalicylic acid method according to Bernfeld (1955).

Polyacrylamide gel electrophoresis and zymogram analysis

Using the method of Laemmli (1970), SDS-PAGE was carried out to determine the probable changes in the quantity of α -amylase and SM0431 protein size marker (Fermentas, Vilnius, Lithuania). SM043 is used as a marker for estimating the position of α -amylase bands in maize having molecular weight between 44.5 – 47.5 kD (Subbarao et al., 1998). Thus, bands corresponding to approximately 45 kD were putatively recognized as α -amylase bands. For zymography, non-denaturing PAGE (10%) was carried out as described by Davis (1964) and electrophoresis was performed at 4 °C. Then the gel was rinsed with deionized water and washed for 45 min at 40 °C in 0.2 M sodium phosphate buffer (pH 7.0). The washed gel was incubated in fresh buffer containing 1% soluble starch at 40 °C for 45 min. After being washed with distilled water, the gel was subjected to staining with Lugol solution at ambient temperature until the appearance of clear zones in protein bands with α -amylase activity against a dark blue background (Liebl et al., 1997).

Ethylene production

For ethylene production experiments, about 5 g of seeds were incubated in 15 cm Petri dishes on two sheets of filter paper (Wathman No 1) moistened with 15 ml treatment solution at 25 °C in darkness for 24 h. Then seeds were rapidly wiped dry on filter paper and transferred to 100 ml glass vials capped with rubber stoppers and incubated in the same conditions for 24 h before ethylene measurement. Ethylene concentration was determined

by withdrawing a sample (1 ml) with a gas-tight syringe and injecting into a gas chromatographer (HP 6890 Series - USA) equipped with a flame ionization detector and a stainless steel column with a Poropak Q 60/80 mesh (2 m in length). The carrier gas was Argon, the column temperature 90 °C, detector temperature 200 °C and injector temperature 120 °C.

RESULTS

Effect of MeJA on seed germination and root growth

As shown in Fig. 1a, seed germination of maize was prevented by increasing MeJA concentrations, especially over 100 μM . Similarly, MeJA inhibited root growth (Fig. 1b). Root length correlated negatively with MeJA concentrations. Treatment with 50 μM MeJA had no significant effect on root growth, but at higher concentrations root growth was significantly reduced.

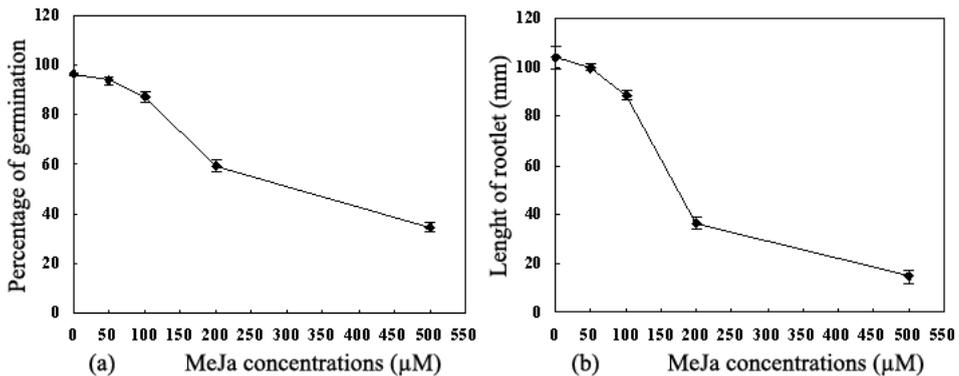


Fig. 1. The effect of different concentrations of MeJA on maize seed germination (a) and root growth (b). The experiments were performed in triplicate and bars represent the SE.

Effect of MeJA on α -amylase activity and content

The activity of α -amylase was characterized in crude extracts and

determined by zymogram analysis after native PAGE which allowed visualization of the enzyme activity in situ. The low concentration of MeJA (50 μM) affected slightly the activity of the enzyme when compared with the control whereas α -amylase activity decreased significantly at concentrations exceeding 100 μM (Fig. 2).

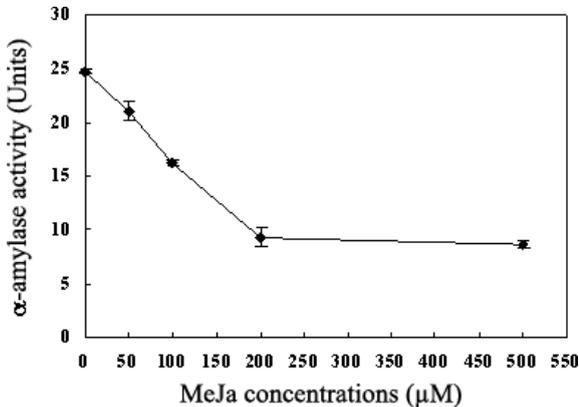


Fig. 2. The effect of MeJA on α -amylase activity during seed germination. One unit of α -amylase is defined as the amount of the enzyme that liberates 1.0 mol of reducing sugar/min with maltose as a standard. The experiments were performed in triplicate and bars represent the SE.

When both PAGE and SDS-PAGE were used for the α -amylase zymogram analysis, it was found that PAGE was more efficient for estimating α -amylase activity in the gel. The light bands indicating the location of α -amylase activity against a dark background are shown in Fig. 3a. The comparison of the band intensity showed that α -amylase activity decreased significantly upon MeJA treatment in the concentration range between 50 and 500 μM . These results correlated well with α -amylase activity in crude extracts. Thus, it can be concluded that the increase in MeJA concentrations from 50 to 500 μM caused a marked decrease in α -amylase activity (Fig. 3a). Further, we examined whether the decrease in α -amylase activity was due to inhibition of the enzyme activity or to reduced enzyme production. SDS-PAGE was employed to answer this question. As shown in Fig. 3b low concentrations of MeJA (50 and 100 μM) caused approximately the same intensity of α -amylase bands as in the control while treatment with higher concentrations of MeJA (200 and 500 μM) reduced the concentration of the enzyme protein.

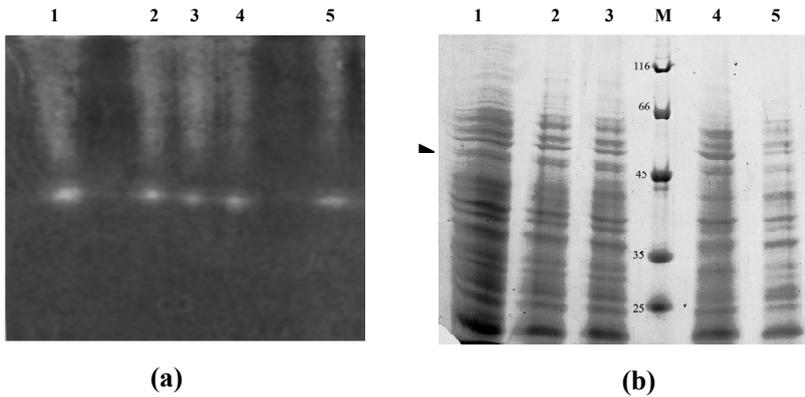


Fig. 3. Native PAGE of crude extracts from maize seeds treated with MeJA and stained for α -amylase activity (a). Lanes 1-5 correspond to samples treated with 0 (control), 50, 100, 200 and 500 μ M of MeJA, respectively. SDS-PAGE of crude extracts from maize seeds treated with MeJA (b). Lanes 1-5 correspond to samples treated with 0 (control), 50, 100, 200 and 500 μ M of MeJA, respectively. Lane M: molecular size marker proteins (kD). The protein markers used (SM0431, Fermentas, Vilnius, Lithuania) for estimating the molecular weight of the bands included lysozyme, 14.2 kD; β -lactoglobulin, 18.4; REase Bsp981, 25.0 kD; lactate dehydrogenase 35.0 kD; ovalbumin, 45.0 kD; bovine serum albumin, 66.2 kD; β -galactosidase, 116.0 kD. The position of the α -amylase band is indicated by an arrow.

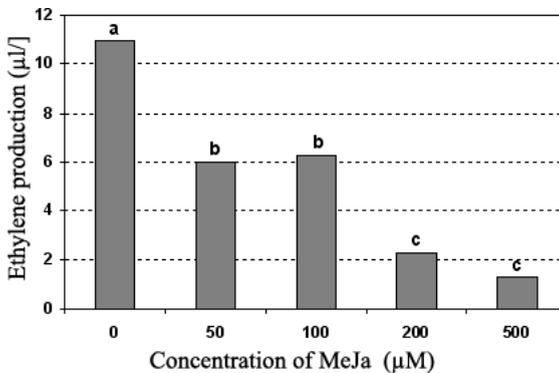


Fig. 4. The effect of MeJA on ethylene production in germinating maize seeds. The means are compared using Duncan's method. Letters above each column indicate statistical differences ($p < 0.01$).

Effect of MeJA on ethylene production during seed germination

MeJA applied at concentrations higher than 50 μ M inhibited germination

significantly (Fig. 1). Rates of germination decreased with increasing MeJA concentration and reached about 40% at 500 μ M. Our results showed that this reduction in the germination rate correlated with reduced ethylene concentration (Fig. 4), suggesting that inhibition of ethylene production was at least one of the causes for germination inhibition caused by MeJA treatment.

DISCUSSION

In the present study, it was clearly shown that MeJA inhibited maize seed germination and root elongation (Fig. 1a,b). The inhibition of root elongation could result from inhibited α -amylase activity, limited ethylene production or changes in both parameters. Our results showed that α -amylase activity decreased with increasing the concentration of MeJA (Fig. 2). α -amylase is a key enzyme in cereal seed germination. It degrades the insoluble starch granules to soluble glucose moieties which translocate towards the embryonic axis. Thus, it can be deduced that the reduced germination percentage and root elongation could be the result of limited production of substrate for respiration and consequently limited energy production. Limited energy production, in turn, results in lower activity of plasma membrane proton-pumping ATPase (H^+ -ATPase) and lower acidification of the cell wall and depolarization and lower ΔpH conditions. Ion absorption will decrease sharply and cell wall loosening will be retarded which consequently results in limited root elongation (Canellas et al., 2002). The above conclusion is further supported by the native-PAGE showing α -amylase activity in polyacrylamide gel (Fig.3a). In this respect our finding was similar to results of other researchers (Bialecka and Kepczynski, 2003a). The reduction of the α -amylase band intensity correlated negatively with MeJA concentrations applied (Fig. 3b). Consequently, it can be proposed that the reduction of germination percentage and decreased root elongation of maize seeds treated with different concentrations of MeJA was due to decreased α -amylase activity as a result of the reduction in α -amylase production in degrading starch reservoirs in the endosperm. The reduction of α -amylase activity due to its limited production could be the consequence of the inhibition of gibberellin

biosynthesis since GA stimulates α -amylase synthesis and its secretion from aleurone layers in cereals.

In our study, ethylene production decreased with increasing MeJA concentrations (Fig. 4). Thus, lower germination percentage due to MeJA treatment can be attributed to lower production of ethylene. It is known that MeJA and ABA are able to inhibit the conversion of ACC to ethylene, and this inhibitory effect increased with increasing the concentration of both growth regulators (Bailly et al., 1992).

In conclusion, our data showed that decreased α -amylase activity and/or concentration as well as reduced ethylene production plays significant roles in the physiological and biochemical processes underlying inhibited seed germination and root elongation in maize under conditions of increased MeJA concentrations.

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