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# Effects of MAPKK Inhibitor PD98059 on Growth of Maize Seedling

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***Authors' contributions***

*This work was carried out in collaboration between all authors and all authors read and approved the final manuscript.*

***Research Article***

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## ABSTRACT

**Aims:** Preliminary observations showed that 75 ìM PD98059 had a long-term effect on growth of maize seedlings. To verify and systemically analyze the effects of 75 ìM PD98059 on growth of maize seedlings, we designed and conducted this experiment.
**Methodology:** We recorded and analyzed the effects of 75 ìM PD98059 on growth of maize seedling during the first fourteen days. The growth traits were observed. The length, fresh weight, and dry weight of shoots or roots, and the root/shoot ratio of fourteen-day old seedlings were measured. Cutting analysis was conducted to analyze the effects of PD98059 on shoot growth.
**Results:** The shoot and root length of control showed about 1.38- and 1.5-fold longer than that of PD98059-treated seedlings, respectively. The shoot and root fresh weight of PD98059-treated seedlings declined to 80% and 79.4% of the control, respectively. The shoot and root dry weight of PD98059-treated seedlings declined to 68.3% and 69.8% of the control, respectively. PD98059 also decreased the length, fresh weight, and dry weight of cuttings.
**Conclusion:** PD98059 had a negative effect on the growth of maize seedlings and this effect was overall on both shoots and roots. The effect of PD98059 on shoot growth seemed to be not due to detrimental effects of PD98059 on roots.

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*Keywords: PD98059; maize seedling; cuttings; growth; MAPK signaling.*

## ABBREVIATIONS

*DMSO: Dimethyl sulfoxide; MAPK: Mitogen-activated protein kinase; MAPKK: MAPK kinase; MAPKKK: MAPK kinase kinase; MPK: MAPK; NACK: NPK1-activating kinesin; NACK-PQR: NACK-NPK1(MAPKKK)-NQK1(MAPKK)-NRK1(MAPK); NPK1: Nucleus- and phragmoplast-localized protein kinase 1; SPSS: Statistical product and service solutions; YODA: One of the MAPKKKs in Arabidopsis.*

## 1. INTRODUCTION

Mitogen-activated protein kinase (MAPK) cascades are universal signaling modules in eukaryotes, including yeasts, animals, and plants [1,2]. A MAPK cascade consists of three consecutively acting protein kinases, MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK [3]. A MAPK pathway, NACK-PQR pathway, has been demonstrated to play a key role in cytokinesis of tobacco cells [4-6]. Recently, it has been shown that, in *Arabidopsis*, the homologues of each constituent of NACK-PQR pathway were also functional in cytokinesis [7-12]. NPK1 is a MAPKKK protein in NACK-PQR pathway. *NPK1*-silenced plants also exhibit overall dwarf phenotype and is involved in N-mediated TMV resistance [13], indicating that NPK1 could initiate a MAPK pathway to regulate plant grwoth. Furthermore, YODA (MAPKKK)-MKK4/MKK5-MPK3/MPK6 regulates stomatal development and patterning in *Arabidopsis* [14-16]. Most recently, YODA-MKK4/MKK5-MPK3/MPK6 cascade was shown to functioned downstream of the ERECTA receptor in regulating localized cell proliferation, which further shaped the morphology of plant organs [17]. Moreover, MAPK cascades are involved in other aspects of plant growth and development, e.g. development of roots [18,19], anther [20], inflorescence and embryo [21], leaf senescence [22], floral organ abscission [23], and shoot development [24]. While MAPK pathways have been studied intensively in *Arabidopsis*, tobacco, and rice, understanding of the roles of MAPK pathway in maize growth and development is still very limited, maybe partially due to the lack of related mutants. PD98059 (2’-amino-3’-methoxyflavone) is a potent, selective, cell-permeable inhibitor of MAPKK by binding its inactive dephosphorylated form, which inhibits the activation of MAP kinase and subsequent phosphorylation of MAP kinase substrates [25,26]. In plants, PD98059 was shown to be functional and was used to investigate the roles of MAPK pathway [27,28]. Previously, we showed that PD98059 affected gravitropism of primary roots of maize [29]. The effects of PD98059 on gravitropism of primary roots were checked in the first three hours. However, we preliminarily found that PD98059 also has a long-term effect on growth rate of maize seedlings. In the study, we recorded and analyzed the effects of 75 μM PD98059 on growth of maize seedling during the first fourteen days.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

PD98059 and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (USA). PD98059 was dissolved in DMSO to produce a stock solution. Then, the stock solution was diluted at least 1,000-fold to achieve the final concentration to ensure that the final DMSO concentration was less than 0.1%. The final concentration of PD98059 used in this study was 75 μM.

### 2.2 Plant Materials and Growth Conditions

Maize cultivar Zhengdan958 (*Zea mays* L. cv Zhengdan 958) was used in this study. Maize seeds were washed several times with tap water and soaked in distilled water for germination. Seedlings with approximately 2 cm primary roots were selected for further treatment. Seedlings were grown in sterile water (control) or sterile water with 75 μM PD98059 (final concentration) with change of sterile water (control) and PD98059 solution (treatment) everyday. The seedlings were cultivated under greenhouse conditions at 22/26ºC (night/day) and 12/12 hours (night/day) for fourteen days prior to sample collection (Fig. 1A). For cutting analysis, five-day old seedlings were excised at the base of the stem. The cut ends of the stems were placed in the beakers wrapped with aluminum foil containing sterile water (control) or sterile water with 75 μM PD98059 (final concentration) for four days with change of sterile water (control) and PD98059 solution (treatment) everyday. Experiments were repeated three times.

### 2.3 Measurements

After treatments of appropriate time, the samples were collected carefully. The shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, cutting height, cutting fresh weight, and cutting dry weight were measured as following. Seedlings (fourteen-day old) were separated into shoots and roots. The length of shoots and roots of seedlings, and the cutting height were measured with a ruler. The shoot length of seedlings and cutting height were measured from the base of the culm to the end of the last leaf. For root length of a seedling, the average value of the lengths of the top ten roots was used. The fresh weight was measured after rapidly drying the surface of shoots, roots, or cuttings. After measuration of fresh weight, the samples were put into envelopes. The envelopes had been dried to a constant weight. All samples were oven-dried at 80ºC for at least 72 h to a constant weight. The dry weight was calculated based on the lost vapor. Four seedlings (and cuttings) were measured as a group. Experiments were repeated five times.

### 2.4 Statistical Analyses

All collected data were subjected to analysis of variance using the general linear model procedure of SPSS (SPSS software version 16.0, Chicago, IL, USA). Standard errors of means were shown in figures. Differences between means were compared by Tukey's HSD test at $P < 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of PD98059 on Growth Traits of Maize Seedlings

In our previous study of the effects of PD98059 on gravitropism of primary roots, we preliminarily found that PD98059 also has a long-term effect on growth rate of maize seedlings [29]. Therefore, we systemically analyzed the effects of PD98059 on growth of maize seedling in this study. As shown in Fig. 1B, after five days, the difference of height and stem color between control and PD98059-treated seedlings had occurred. The growth of shoot or root was retarded by PD98059. Notably, the stems of most PD98059-treated seedlings showed to be purple, which was not observed in control, indicating that it was likely that PD98059 may regulate the biosynthesis of anthocyanidin by inhibiting MAPKK. However, the color trait of PD98059-treated seedlings only occurred in the first week (around six days). As shown in Fig. 1C, when we checked the nine-day old seedlings, no obvious difference in color was observed between PD98059-treated seedlings and control, whereas retardant growth of PD98059-treated seedlings was evident. These results showed that treatment with 75 µM PD98059 altered the stem color of maize seedlings in the first week (around six days) and had a negative effect on the growth of maize seedlings.

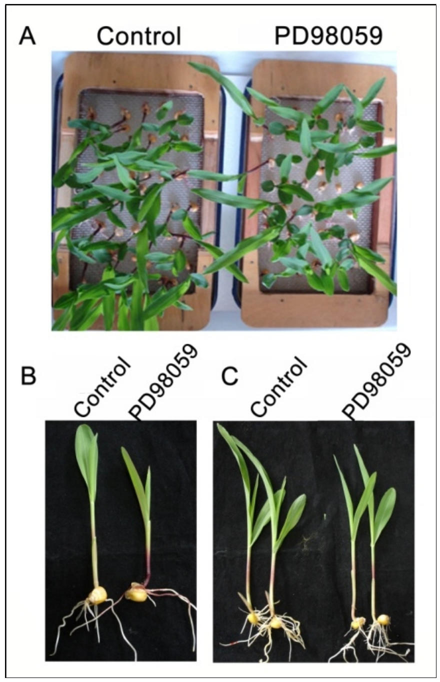


**Fig. 1. Comparison of seedlings in control and PD98059-treated seedlings: (A) Overview of the experimental design. (B) Comparison of stem color of five-day old seedlings. (C) Comparison of nine-day old seedlings**

*Seedlings were grown in sterile water (control) or sterile water with 75 µM PD98059 (final concentration) with change of sterile water (control) and PD98059 solution (treatment) everyday. The seedlings were cultivated under greenhouse conditions at 22/26°C (night/day) and 12/12 hours (night/day) for fourteen days prior to sample collection. Experiments were repeated three times. Control: control check; PD98059, PD98059-treated seedlings.*

## 3.2 Effect of PD98059 on Plant Height and Root Length

Since PD98059 had a negative effect on the growth of maize seedlings, we measured the shoot length and root lenght. In our study, the average value of the lengths of the top ten roots was used to determine the root length of a seedling. Fourteen-day old seedlings were used. As shown in Fig. 2, the shoot length of control was about 22 cm, whereas the shoot length of PD98059-treated seedlings was about 16 cm, indicating PD98059 significantly retarded the shoot growth of maize seedlings. The results of root length showed the same trend. The root length of control showed about a 1.5-fold longer than that of PD98059-treated seedlings (about 12 cm versus about 8 cm). These results showed that treatment with 75 µM PD98059 had a significantly negative effect on the growth of both shoots and roots of fourteen-day old seedlings.

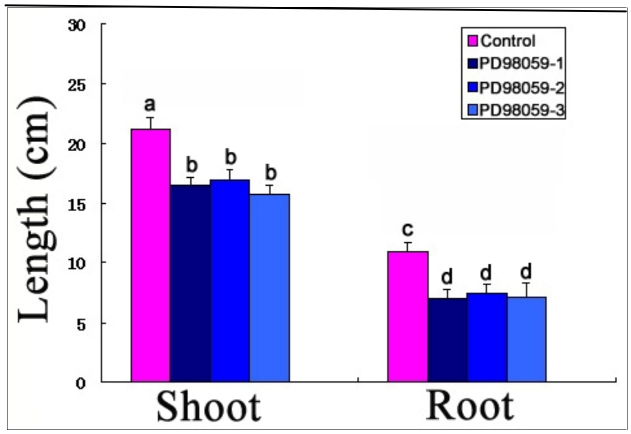


**Fig. 2. Effect of PD98059 on shoot and root length of fourteen-day old seedlings**

*The shoot length of control was about 22 cm, whereas the shoot length of PD98059-treated seedlings was about 16 cm. The root length of control was about 12 cm, whereas the root length of PD98059-treated seedlings was about 8 cm. Control: control check; PD98059, PD98059-treated seedlings.Bars labeled with the same letter are not statistically significantly different (P<0.05).*

### 3.3 Effect of PD98059 on Fresh and Dry Weight

Fresh weight and dry weight are important considerations for plant growth. In order to further determine the effects of PD98059 on the growth of both shoots and roots of maize seedlings, we measured the fresh weight and dry weight of shoots or roots. In our study, fourteen-day old seedlings were used and four seedlings were measured as a group (Fig. 3A). As shown in Fig. 3B, the shoot fresh weight (four seedlings) of control was about 5 gram, whereas the root fresh weight was about 6.3 gram. Treatment with 75 µM PD98059 could decrease the fresh weight and dry weight of fourteen-day old seedlings. The shoot fresh weight of PD98059-treated seedlings was about 4 gram and declined to about 80% of the control. Retardarce of roots in PD98059-treated seedlings seemed to be more severe than that of shoots. The root fresh weight of PD98059-treated seedlings declined to 79.4% of the control, i.e. the root fresh weight was around 5 gram. Furthermore, the shoots and roots were oven-dried at 80ºC followed by mensuration of dry weight. As shown in Fig. 3C, comparison of the dry weights of shoots or roots between control and PD98059-treated seedlings showed the same trend as the comparison of fresh weight. The shoot dry weight and root dry weight of control were about 4.4 and 5.3 gram, respectively. The shoot dry weight of PD98059-treated seedlings declined to 68.3% of the control, and the root dry weight of PD98059-treated seedlings declined to 69.8% of the control. To study whether the retardarce of shoot growth was due to detrimental effects of PD98059 on roots, the root/shoot ratio in control or PD98059-treated seedlings was calculated. As shown in Fig. 3D, although the average value of the root/shoot ratio in PD98059-treated seedlings was slightly larger than that in control, there was no significant difference was observed, indicating that treatment with 75 µM PD98059 in maize roots inhibited the growth of both roots and shoots. Overall, the results in Fig. 3 showed that treatment with 75 µM PD98059 affected fresh weight and dry weight of both shoots and roots of maize seedlings.

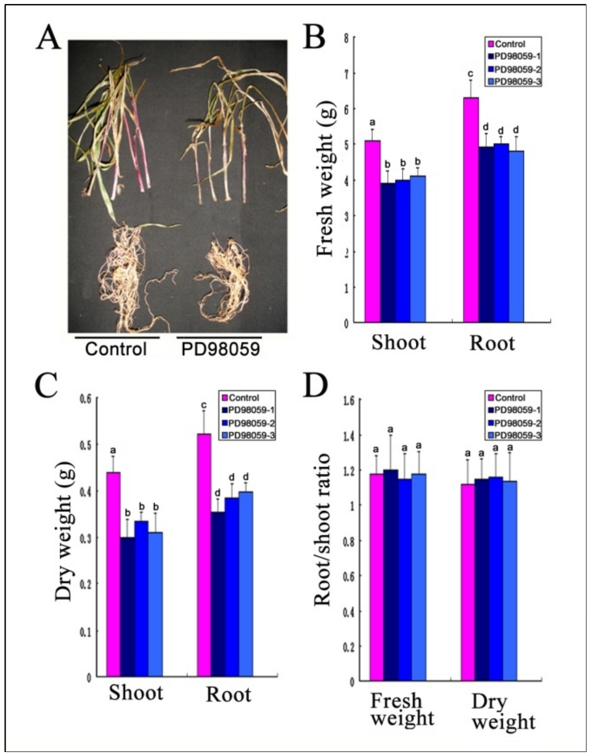


**Fig. 3. Effect of PD98059 on fresh weight and dry weight of shoots or roots, and the root/shoot ratio of fourteen-day old seedlings**

*A. Photograph of oven-dried shoots and roots. B. Effect of PD98059 on fresh weight of shoots or roots of fourteen-day old seedlings. C. Effect of PD98059 on dry weight of shoots or roots of fourteen-day old seedlings. D. Effect of PD98059 on the root/shoot ratio of fourteen-day old seedlings. Control: control check; PD98059, PD98059-treated seedlings. Bars labeled with the same letter are not statistically significantly different (P<0.05).*

## 3.4 Effects of PD98059 on Cuttings

Although the results in Fig. 3 showed that PD98059 had an overall effect on both shoots and roots, it seemed that the effects were not even. To further determine whether the retardarce of shoot growth was due to detrimental effects of PD98059 on roots, we conducted cutting analysis (Fig. 4). Five-day old seedlings were excised at the base of the stem. Trimmed seedlings with 5 cm height were used. After treatment with 75 μM PD98059 for four days, the shoot height, fresh weight, and dry weight were measured. As shown in Fig. 4A, the control could grow to about 7 cm, whereas PD98059-treated cuttings could only grow to about 5.5 cm. The fresh weight and dry weight of 5-cm-height cuttings (four cuttings as a group) were about 0.4 g and 0.033 g, respectively (Fig. 4B,C). After four days, the fresh weight of control was about 0.59 g, and the dry weight was about 0.044 g (Fig. 4B). After four days, the fresh weight and dry weight of PD98059-treated cuttings were about 0.45 g and 0.0358 g, respectively (Fig. 4B,C). These results indicated that treatment with 75 μM PD98059 affected growth (height, fresh weight, and dry weight) of maize cuttings, and further indicated that the retardarce of shoot growth in seedling analysis was not due to detrimental effects of PD98059 on roots.

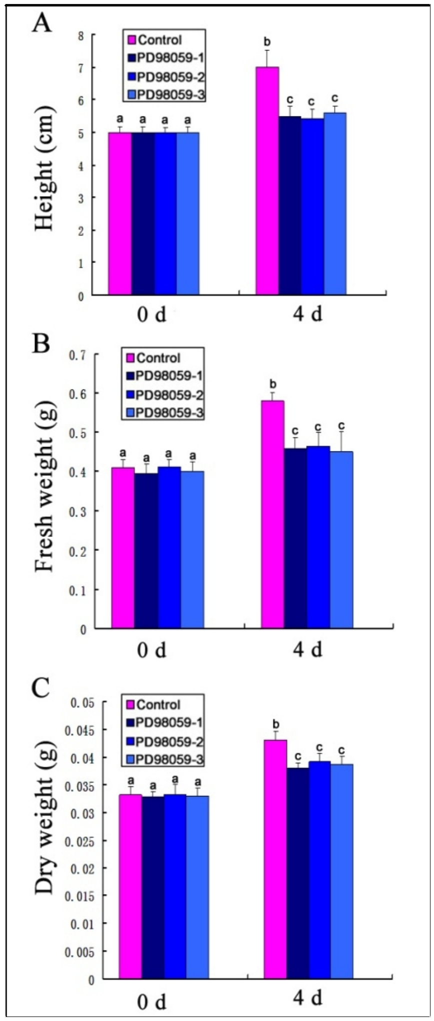


**Fig. 4. Effect of PD98059 on height, fresh weight and dry weight of cuttings**

*Five-day old seedlings were excised at the base of the stem. Trimmed seedlings with 5 cm height were used. After treatment with 75 ìM PD98059 for four days, the shoot height (A), fresh weight (B), and dry weight (C) were measured. Control: control check; PD98059, PD98059-treated seedlings. Bars labeled with the same letter are not statistically significantly different (P<0.05).*

## 4. CONCLUSION

In this study, we recorded and analyzed the long-term effects (two weeks) of 75 μM PD98059 on growth of maize seedling based on preliminary observations in our study of gravitropism. PD98059 altered the stem color (Fig. 1B) of maize seedlings in the first week (around six days) and had a negative effect on the growth of maize seedlings (Fig. 1C and 2). Treatment with PD98059 decreased the length, fresh weight, and dry weight of shoots or roots of fourteen-day old seedlings (Fig. 2 and 3). PD98059 had an overall effect on both shoots and roots, as no significant difference was observed between the root/shoot ratios of control and PD98059-treated seedlings (Fig. 3D). Cutting analysis showed that the effect of PD98059 on shoot growth was not due to detrimental effects of PD98059 on roots, as

PD98059 decreased the length, fresh weight, and dry weight of cuttings. Overall, 75 μM PD98059, maybe by inhibiting the activities of MAPKK and thereby the MAPK pathway, negatively regulates the growth of maize seedlings.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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