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Ethylene participates in the brassinolide-regulated asymmetric growth of *O. sativa* root

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ABSTRACT

In this work, brassinosteroid (BR)-induced root asymmetric growth as well as its interaction with ethylene (ETH) were investigated. The results reveal that 2, 4-epibrassinolide (2,4-eBL) can induce asymmetric growth of the primary roots of monocotyledonous *Oryza sativa* to form root coils and waves under both illumination and dark conditions. Similarly, ETH can also induce asymmetric growth of the primary roots of *O. sativa* to form hooked structures. Upon adding a concentration of 2×10^{-8} M 2,4-eBL and inhibitors of ethylene simultaneously, silver thiosulfate (STS) and aminooxyacetic acid could completely eliminate the 2,4-eBL-induced asymmetric growth of roots, while the asymmetric growth could also be partly eliminated by $CoCl_2$. Meanwhile, the effect of ETH inhibitors was proven to be reversible, wherein 2,4-eBL can exert its function and induce the asymmetric growth of *O. sativa* roots after the removal of STS. These conclusions provide a scientific foundation for further investigations of the relationships between BR and ETH.

1. Introduction

Brassinosteroids (BRs) are natural products extensively distributed in plants, the structure of which is similar to insect and animal steroid hormones (Konishi and Komatsu 2003). Brassinolide (BL) was first isolated from *Brassica napus* L. powder by Grove et al. (1979). Since then, over different types of BRs have been identified (Grove et al. 1979; Oklestkova et al. 2015). BRs have extensive physiological effects on processes including seed dormancy and germination, stem elongation, photomorphogenesis, leaf unrolling, root growth, vascular differentiation, flowering, senescence and gravitropic response (Bishop and Yokota 2001; Shimada et al. 2003; Gudesblat and Russinova 2011; Vriet et al. 2012). To date, BRs have been discovered in the roots of *Zea mays* L, *Arabidopsis thaliana* (L.) Heynh, *Pisum sativum* and *Lycopersicon esculentum* Mill (Kim et al. 2000; Yokota et al. 2001; Bancosİ et al. 2002; Shimada et al. 2003). Research has verified that BRs play important roles in regulating root length, root meristem size, root hair formation and lateral root initiation (Vragović et al. 2015; Wei and Li 2016). In *Arabidopsis thaliana* (L.) Heynh., BRs can promote root elongation at extremely low concentrations (≤0.1 nM), while BRs will suppress root elongation at high concentrations (Müssig et al. 2003; González-García et al. 2011). Precise tissue-specific translatome profiling also indicates that BR signals produce dual effects in regulating the root meristem. Specifically, BRs can promote and suppress differentiation of the root meristem, depending on whether the BR signals are located in the epidermis or in the stele (Vragović et al. 2015; Salazar-Henao et al. 2016).

Ethylene (ETH) is one of the first discovered plant hormones and can regulate numerous physiological processes during plant growth and development, such as seed germination, root hair elongation, flower senescence, fruit ripening, and tolerance to biotic and abiotic stresses (Wang et al. 2002; Anderson et al. 2004). The biosynthesis of ETH is regulated via successive enzymatic reactions. Methionine (Met) is first converted to S-adenosyl-Met. Secondly, ACC synthases (ACS) convert the S-adenosyl-Met into 1-aminocyclopropane-1-carboxylic acid (ACC), and finally, ACC oxidase (ACO) converts ACC into ETH (Adams and Yang 1979; Chen et al. 2017). During this process, aminooxyacetic acid (AOA) serves as an analogue of the ACS prosthetic group pyridoxal-5′-phosphate (PLP) which can competitively bind and deactivate zymoprotein, thus inhibiting ETH synthesis. Typically, AOA is a specific inhibitor of ETH biosynthesis (Yang and Hoffman 1984; Spollen et al. 2000; Wang et al. 2009). Co^{2+} can suppress ACO and, thus, restrain the conversion of ACC to ETH (Lau and Yang 1976; Romera and Alcantara 1994). Additionally, Ag^{+} is an ETH perception antagonist (Chen et al. 2017). ETH receptors contain binding sites for cuprous ions (Cu^{+}),

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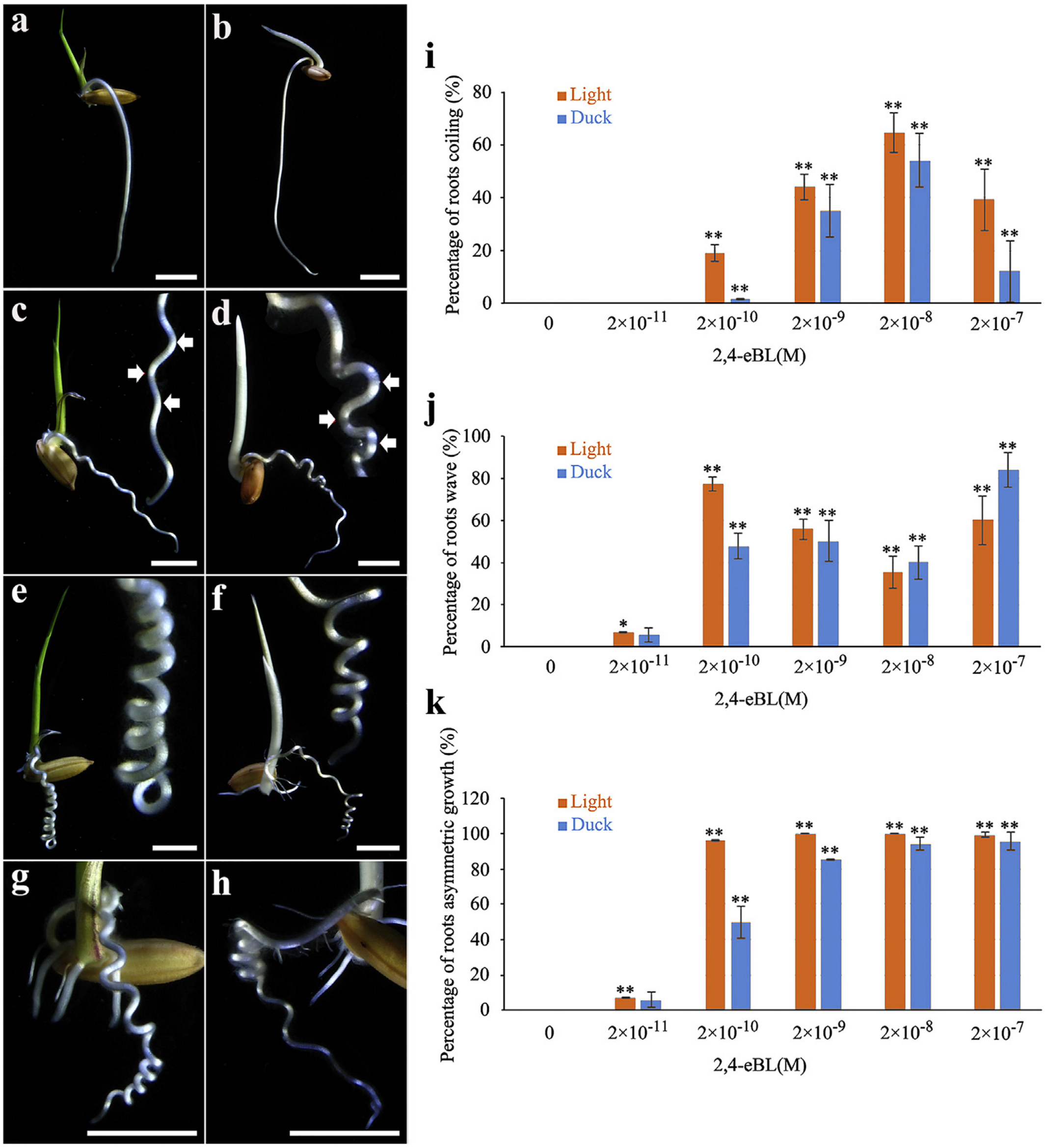

Fig. 1. 2,4-eBL induced the asymmetric growth of O. sativa primary roots. (A and B) Natural growth of O. sativa primary roots; (C and D) wave formation in O. sativa primary roots with 2×10^{-8} M 2,4-eBL; (E and F) coiling formation in O. sativa primary roots with 2×10^{-8} M 2,4-eBL; (G) wave formation before coiling formation in O. sativa primary roots with 2×10^{-8} M 2,4-eBL; (H) coiling formation before wave formation in O. sativa primary roots with of 2×10^{-8} M 2,4-eBL; (I, J, and K) percentage of 2,4-eBL-induced asymmetric growth of O. sativa primary roots. The culture time was 7 d. (A, C, and E) Culture in illumination; (B, D, and F) culture in dark. Bars = 5 mm.

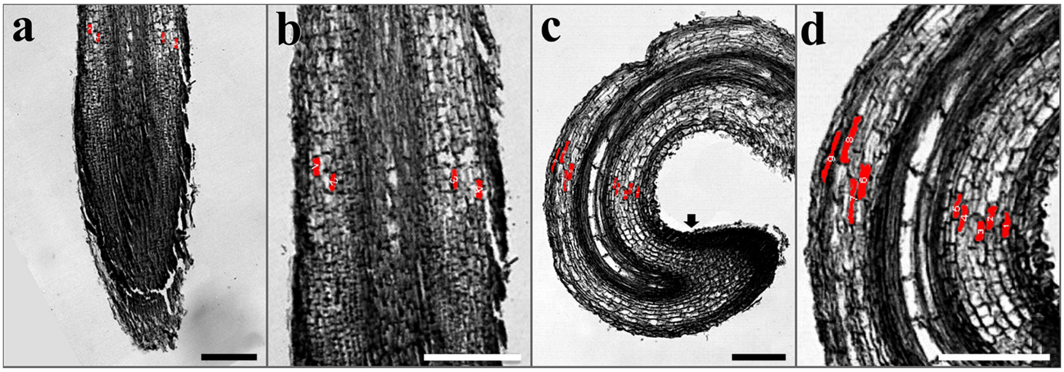

Fig. 2. Observation of O. sativa primary root microsections. (A and B) Normally growing O. sativa primary roots; (C and D) coiling formation in O. sativa primary roots under the action of 2×10^{-8} M 2,4-eBL. Bars = 200 μm.