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Effect of photoperiod on the formation of cherry radish root

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ABSTRACT

Radish root formation under short photoperiod is a common sense, however, cherry radish is a kind of four-season radish and the relation of photoperiod and cherry radish root formation is not clear until now. Effect of different photoperiods (8 h/16 h, 12 h/12 h, and 16 h/8 h) on the development and antioxidant ability of cherry radish as well as expression of relative genes was investigated in this study. Surprisingly, the most robust radish plant and largest biomass were found at the long photoperiod regime (16h/8 h), even though both the polyphenol content and antioxidant ability in terms of DPPH and FRAP were lower than those in 12/12 h photoperiod. Moreover, under 16h/8h photoperiod it can form the radish product organs in 30 d but not for the others. Gene expression analysis showed that eight non-coding miRNAs and coding RNAs were both involved in the regulation of radish root formation. MiR160, miR165, and miR396 were up-regulated by 16h/8 h photoperiod treatment while miR156 and miR157 were down-regulated. The up-regulation of miR160 and the opposite expression trend of ARF16 indicated that miR160 may mediate the light-respond root formation by targeting ARF16. An auxin response factor ARF6 and a cell wall structure related *WRKY2* were also significantly up-regulated by the longer illumination treatment compared with the control. Thus, unlike other radish roots that form in short-day condition, the cherry radish roots grow fastest in long photoperiod and miR160-ARF16 are involved in the quick formation of cherry radish root.

1. Introduction

Radish (*Raphanus sativus*) is an edible root vegetable of the Brassicaceae family, with its root and hypocotyl as storage organs of nutrients (Hall, 1990). There are many types of radishes which differ in size and color (Kaymak et al., 2016). The diameter of a mature radish root ranges from less than 1 cm to more than 30 cm (Mitsui et al., 2015). The cherry radish is a type of four-season radish with a tuberous root ∼4 cm in length tuberous root.

MicroRNAs (miRNAs) play key roles in the processes of plant growth and development. Several miRNAs, including miR156, miR160, miR164, miR165, miR167, and miR396, have been proved to be involved in root development (Table 1). The evolutionarily conserved miR156 is expressed highly in seedlings, and its levels decrease with age (Wang et al., 2009). The expression level of *miR156a* is correlated with the number of lateral roots. MiR156 regulates the *SQUAMOSA--PROMOTER BINDING PROTEIN-LIKE (SPL)* genes (*SPL3*, *9*, and *10*) to suppress lateral root growth (Yu et al., 2015). In addition to the conserved SPL targets, miR156 cleaves a WD-40 mRNA in the root apices of *Medicago truncatula* (Naya et al., 2010), indicating a potential role for miR156/WD-40 in root development (Wang et al., 2015). MiR160 targets *ARF10*, *16*, and *17* to affect root cap development. Plants with overexpressed *miR160c* produce shorter roots, and a similar phenotype was observed in *arf10/arf16* double mutants (Wang et al., 2005). The module *miR164–NAC1* is believed to transduce auxin signals for lateral root development (Guo et al., 2005; Yu et al., 2015). MiR165/166 are involved in embryonic root development through their targeting of *PHABULOSA* and *PHAVOLUTA* (Khan et al., 2011), miR167–IAR3 (for IAA-Ala Resistant 3) can affect the development of lateral roots by regulating the IAA levels (Kinoshita et al., 2012). MiR396 interacts with Growth-Regulating Factors to regulate the reprogramming of root cells (Rodriguez et al., 2015). In *Arabidopsis thaliana*, the overexpression of *AtMIR396a* resultes in shorter roots and the miR396a-mediated *bHLH74* repression helps regulate root growth (Bao et al., 2014).

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Table 1
miRNAs as regulators of root development and architecture.

miRNA	Sequence 5'-3'	Target Gene	Function in Root	Refs.
miR156	UGACAGAAGAGAGUGAGCAC	SPL3, SPL9, SPL10	lateral root growth	(Yu et al., 2015)
		WD40 transducin-like protein SWA1	root growth	(Naya et al., 2010)
miR160	UGCCUGGCUCCCUGUAUGCCA	ARF10, ARF16, ARF17	primary root growth, primary root density, and gravitropism	(Wang et al., 2005)
miR164	UGGAGAAGCAGGGCACGUGCA	NAC1	Lateral root development	(Guo et al., 2005)
miR165	UCGGACCAGGCUUCAUCCCCC	PHB, PHV	Root cell fate	(Carlsbecker et al., 2010)
miR167	UGAAGCUGCCAGCAUGAUCUA	IAR3	lateral root development	(Kinoshita et al., 2012)
miR396b	UUCCACAGCUUUCUUGAACUU	GRF	Promotion of cell proliferation, Regulator in the Reprogramming of Root Cells during Cyst Nematode Infection	(Rodriguez et al., 2015, Hewezi et al., 2012)

Photoperiod is a light factor that influences the growth and development of plants, including their roots. Studies on photoperiodism indicate that the formation of tubers commonly results from short-day conditions (Plitt, 1932). The short-day (8 h) period, independent of low temperature (4 °C) or moderate temperature(18 °C), is best for radish growth, resulting in a larger shoot:root ratio and greater specific leaf area (Sirtautas et al., 2011). Photoperiod changes not only affect plant morphology but also influence their quality. A number of flavonoids, including anthocyanin, catechins, flavonols, hydroxycinnamic acids, and hydroxybenzoic acids, as well as genes involved in flavonoid biosynthesis, in sweet potato (*Ipomoea batatas*) leaves were dramatically induced after exposure to a long-day photoperiod (16 h light) compared with those exposed to a short-day photoperiod (8 h light) (Carvalho et al., 2010). The continuous exposure to LED light for 24 h significantly enhanced the free-radical scavenging activity and increased phenolic compound concentrations in lettuce (*Lactuca sativa*) (Bian et al., 2016).

Although both miRNA and photoperiod influence root development, little is known about their functions during radish root formation. Short-day illumination may be beneficial because nutrients are transferred into storage organs. In our experiment, three regimes of light and dark conditions, 8 h light/16 h dark, 12 h light/12 h dark, and 16 h light/8 h dark, were used to investigate the effects of photoperiod on the growth and development of cherry radish, and, subsequently, the expression levels of genes, including miRNAs and their targets, were analyzed to elucidate the possible molecular mechanism of root expansion.

2. Materials and methods

2.1. Plant material

Seed of cherry radish (*Raphanus sativus* L. var. 'Changfeng') was purchased in the Lvdinongzi seed company in Fuzhou. Seeds were planted in perlite with water in 15-cm petri dish at 28 ℃ (dark). After 1 day, the radicle came out and the germinated seedlings were chosen to grow in the incubator (12 h light/12 h dark) for 10 days. Then 10-day-old seedlings were transferred into the container (51 cm × 38 cm × 15 cm) with 3 L nutrition solution (945 mg/L $Ca_2NO_3{\cdot}4H_2O$, 809 mg/L K_2NO_3, 153 mg/L KH_2PO_4, 493 mg/L $MgSO_4{\cdot}7H_2O$, 30 mg/L EDTA $FeNa_2$, 2.86 mg/L H_3BO_3, 2.13 mg/L $MnSO_4$, 0.22 mg/L $ZnSO_4$, 0.08 mg/L $CuSO_4$, and 0.02 mg/L $(NH_4)_2MoO_4$) under different photoperiods (16 h light / 8 h dark, 12 h light / 12 h dark, and 8 h light / 16 h dark). The light intensity for each treatment was set to 150 μmol/m^2/s and the light quality ratio of red and blue LED is 8:2. There were twelve plants per container and three replicates for each treatment. The height, width, and biomass of seedlings were measured every five days. The aerial and underground thicken part were harvested at day 30 from the plants in three different photoperiod conditions and immediately frozen in liquid nitrogen and stored at -80 ℃ for analysis of total polyphenol content, antioxidant ability, and relatively gene expression level.

2.2. Extractions for total polyphenols and antioxidant capability

Radish extracts for total polyphenols and antioxidant capability were prepared followed the method of Thaipong (Thaipong et al., 2006) with some modifications. Tissues of 0.3 g from leaves or roots were homogenized by 1.6 mL methanol. The homogenates were kept at 4 ℃ for 12 h in the dark and then centrifuged at 15,000 rpm for 20 min. The supernatants were collected and stored at -20 ℃ until analysis.

2.3. Assay of total polyphenol content

Phenolic compounds were determined using Folin–Ciocalteu reagent method. 0.5 mL supernatants and 2.5 mL 10% Folin–Ciocalteu reagent were combined in a centrifuge tube and then mixed well using a Vortex. The mixture was allowed to react for 5 min then 2 mL of 7.5% Na_2CO_3 solution was added and mixed well. The solution was incubated at 25 ℃ in the dark for 2 h. The absorbance was measured at 765 nm with a UV–Vis spectrophotometer (UV 5100H, METASH. Shanghai, China). Gallic acid was used as a standard and the results were expressed as milligrams (mg) of gallic acid equivalent (GAE) per dw (Thaipong et al., 2006; Guo et al., 2011).

2.4. Analysis of antioxidant ability

For FRAP assay, the procedure followed the method of Benzie and Strain (1996) with some modifications (Benzie and Strain, 1996). The FRAP reagent included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mM HCl, and 20 mM ferric chloride ($FeCl_3{\cdot}6H_2O$) in the ratio of 10:1:1 (v/v/v). 0.3 mL supernatants were allowed to react with 2.7 mL of the FRAP working solution incubated at 37 ℃ for 10 min and the absorbance was then recorded at 593 nm using a UV–Vis spectrophotometer (UV 5100H, METASH. Shanghai, China). FRAP values were calculated from $FeSO_4{\cdot}7H_2O$ standard curves and expressed as mM Fe^{2+} per dw.

The $ABTS^{\cdot+}$ assay was done according to the method of Arnao et al. (2001) with some modifications (Arnao et al., 2001). The $ABTS^{\cdot+}$ solution were prepared by mixing 7.5 mM $ABTS^{\cdot+}$ solution and 2.6 mM $K_2S_2O_8$ in the ratio of 1:1 (v/v) and reacted at 25 ℃ for 12 h in the dark. The working solution was obtained by adding 1 mL $ABTS^{\cdot+}$ solution to 33 mL methanol to reach an absorbance of 1.1 ± 0.02 units at 734 nm (Thaipong et al., 2006). 0.15 mL supernatants were allowed to react with 2.85 mL of the $ABTS^{\cdot+}$ working solution incubated for 2 h and the absorbance was then recorded at 734 nm using a UV–Vis spectrophotometer (UV 5100H, METASH, Shanghai, China). The standard curve was drawn from the data of different concentrations of Trolox (25 and 800 mM) reacted with $ABTS^{\cdot+}$ working solution. $ABTS^{\cdot+}$ values were expressed in mM Trolox equivalents (TE) per dw.

The DPPH assay was done according to the method of Brand-Williams et al. (1995) with some modifications (Brand-Williams et al., 1995). The DPPH solution was prepared by dissolving 24 mg DPPH with 100 mL ethanol. The solution was then diluted by methanol to

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