Characterization of a novel mutant with inhibition of storage root formation in sweet potato†

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Sweet potato is one of the most important food crops and is cultivated worldwide. Storage root (SR) is an economically important component of sweet potato, and the primary target of sweet potato breeding is its SR traits and breeding objectives such as good eating quality, resistance to diseases and pests, and high yield. The development of SR begins from adventitious fibrous roots derived from the stem nodules. These roots enlarge to form starch-containing pencil roots and SR. Although many studies have been conducted on the mechanism of its storage root formation, the details have not yet been fully clarified.[1] Mutants on SR formation will aid in elucidating the mechanism. In a previous study, we screened a mutant line of C20-8-1 from cabon-ion beam-irradiated lines.[2] During the mutant screening process, C20-8-1 exhibited a decrease in DNA content and inhibition of SR formation. To elucidate the details of the inhibitory mechanism of SR, we characterized the total phenotype of C20-8-1 mutant line and analyzed the gene expression involved in the regulation of SR formation.

The heavy-ion beam-irradiated line C20-8-1 was derived from *Ipomoea batatas* 'Beniharuka.' *In vitro* cultured C20-8-1 and wild type (WT) of 'Beniharuka' were transplanted into 10 L of black plastic pots filled with culture soil and grown in a greenhouse at the University of Miyazaki. The shoots and roots of each plant were harvested 15, 45, and 90 days after transplanting (DAT).

The total plant weight and shoot weight in C20-8-1 did not change from those of WT, and remarkable growth inhibition was not observed. However, the yield and SR number in C20-8-1 decreased significantly compared with those in WT. After the results of the yield, the SR formation process in C20-8-1 were observed. The morphological classification of sweet potato roots was based on their developmental stages, according to Wang *et al.*[3] At 15 DAT, all the roots were categorized as early fibrous roots, with a diameter <2 mm without anthocyanin accumulation, in WT and C20-8-1 plant. At 45 DAT, the roots in WT began to swell, and late-developing pencil roots (5 mm $\leq$ diameter $\leq$ 20 mm) were observed. However, in C20-8-1, all the roots were fibrous roots and several early-developing pencil roots (2 mm $\leq$ diameter $<$ 5 mm). When we investigated the proportion of the roots in the late fibrous root stage and later at 90 DAT, the late-developing pencil roots significantly decreased at 90 DAT in C20-8-1 (Fig. 1). The induction of late fibrous roots was not inhibited in C20-8-1. Therefore, the key process for the inhibition of SR formation in C20-8-1 can be interpreted as a transition from fibrous roots to pencil roots.

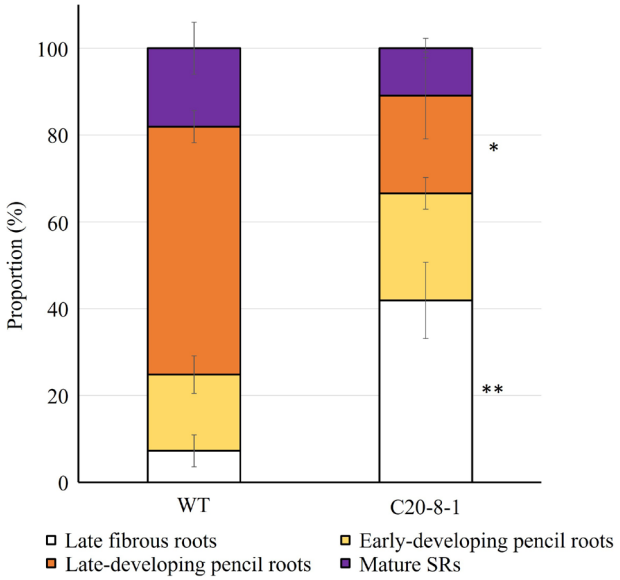

Fig. 1. Proportion of roots in the developmental stages at 90 DAT. Each value shows the mean ± S.E. *Value of C20-8-1 differs significantly from that of WT in each developmental stage according to the t-test ($n = 3$, *: $p < 0.05$, **: $p < 0.01$).

We focused on the early development of SR in C20-8-1, and qRT-PCR was used to analyze the SR development-related gene expression levels. The upregulation of starch biosynthesis-related genes and downregulation of lignin biosynthesis genes with SR swelling were not confirmed in the root of C20-8-1 during the developmental transition stage, suggesting that most of the roots in C20-8-1 are in the pre-transition state toward the SR swelling. Gene deletions associated with the decrease in DNA content in C20-8-1 may be considered to inhibit SR formation. The knockout of the key genes mentioned above was not observed in C20-8-1. Further clarification of the details of the mutation in the genome and identification of the gene responsible for the phenotype is expected to provide new insights into SR formation.

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