Phenotypic analysis of transgenic *Arabidopsis* with 2-Oxoglutarate dependent deoxygenase like gene, *CuAOP1*, isolated from Satsuma mandarin ‘Aoshima’ and HPLC analysis of glucosinolate.

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**[Purpose]**
Two proteins catalyze the essential role of oxygenation and hydroxylation reactions in different biosynthetic processes in all the living organisms, namely, cytochrome P450 monoxygenase (CYP) along with a soluble, non-heme iron-containing protein-2-oxoglutarate dependent dioxygenase (2ODD). 2ODD is responsible for both gibberellin biosynthesis and catabolism (GA oxidase genes) and Glucosinolate metabolism (AOP genes) (Kawai et al. 2014). There are three AOP genes among which AOP2 catalyzes in the transformation of methylsulfinylalkyl glucosinolates to alkenyl glucosinolates, and AOP3 catalyzes the conversion of 3–methylsulfinylpropyl glucosinolate to 3–hydroxypropyl Glucosinolate. In previous years, many GA biosynthetic genes were isolated from satsuma mandarin ‘Aoshima unshiu’ and analyzed to clarify their functions. CuAOP1 is one of them and related to 2ODD and more precisely, homologous to AOP1 groups. Nevertheless, the function of AOP1 gene in *Aoshima* is yet to be clarified. This phenotypical study was conducted to ensure the gene was manipulating the growth of the transgenic *Arabidopsis*.

**[Materials and Methods]**
The transformation of *Arabidopsis thaliana* was performed according to Clough and Bent, 1998, using the floral dip method, *Agrobacterium tumefaciens* strain EHA101 containing a binary vector 35Ω::CuAOP1. The collection, sterilization, sowing on ½ MS media containing Kanamycin and Cefotaxime, stratification at 4°C, transplantation to vermiculite and perlite 1:1, cultivation at 22°C in long day condition, fertilization with Hyponex 0.1% solution and regeneration of seeds were done up to 3 generations according to Kotoda et al. 2017. From 24 lines in the T1 generation, five lines were continued up to T 3 to observe the phenotype. An empty vector PSMAK251 transgenic line was considered as the control. Phenotypic data, such as days to bolting and flowering, number of rosette and cauline leaves, the height of inflorescence, number of secondary shoots, the average length of internodes, were observed in 12 plants from each line of T 3 generation. Glucosinolates were extracted from the T4 generation L4, L6, L7 lines selected based on the height of inflorescence and analyzed with HPLC (Grosser and Van Dam 2017).

**[Result and Discussion]**
L7 and L9 showed the most delayed flowering during growth. L6 and L7 showed the highest number of rosette leaves at bolting, flowering and at 30th day, which was statically similar to the control. The regression equation showed that the L9 had the lowest increasing trend of the number of leaves compared to other transgenic lines and controls. It showed the least number of cauline leaves and significantly different from the control and other transgenics. The height of the primary inflorescence of L9 was found to be the shortest in both 30th and 40th days, and the growth rate was also the least (Figure 1) with the least number of branching which was followed by L4. L6 was observed with the highest average length of internodes, followed by L7, L1, and L9, but L4 showed the least average length of internodes. L9 had the smallest number of pods, statistically similar to L6 and L7. The length of randomly selected 20 pods was taken from each line, and the average was calculated. Tukey’s HSD test revealed that L1, L6 and L7 to be statistically similar to the control, while L4 and L9 to have significant difference with control. Depending on the phenotypic result we investigated the reason for decreasing vigor of the transgenic L4 and L9.

From the Glucosinolate analysis with HPLC, it was clear that the total glucosinolate was increased in L4 (1.46 mg/gdw) and L9 (1.14 mg/gdw) transgenic lines compared to control (0.73 mg/gdw). To be more precisely, glucobrassicin and 4-methoxyglucobrassicin was increased in L4 and L9 and showed a significant difference with the control. Nevertheless, glucoraphanin showed no significant difference in L4, L9 and control, though the level was increased. The results from above experiments indicate that the AOP1 gene from *Aoshima* overexpressed in *Arabidopsis* increased the glucosinolate biosynthesis (Figure 2). In future we have a plan to find out whether there is any relationship between gibberellins and glucosinolates in the transgenics.

![Figure 1: Mean comparison of heights of inflorescences at 30th and 40th days.](image1.png)

![Figure 2: Analysis of glucosinolates in transgenic *Arabidopsis* by HPLC.](image2.png)