論文の内容の要旨

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Studies on the diversity of *Portulaca umbraticola* cultivars

（ハナスベリヒユ園芸品種の多様性に関する研究）

In the genus *Portulaca* two species are mainly used for ornamental purpose that is *P. grandiflora* and *P. umbraticola*. The flower shape of *P. umbraticola* resembles that of *P. grandiflora*, and its leaf morphology highly resembles that of common purslane (*P. oleracea*). As a result, *P. umbraticola* cultivars were named as ‘Hana-Suberihiyu’ in Japanese, which means ornamental *P. oleracea* or were simply called as ‘Portulaca’, which is its genus name. Ornamental *Portulaca* has recently become an important summer bedding plant in Japan due to its adaptability to the hot and dry weather conditions a typical of the Japanese summer. Recent trends show an increase in the consumers of ornamental *Portulaca*, with *Portulaca* being ranked as the 9th most grown bedding plant in Japan as of 2009. It was estimated that over 70% of the ornamental *Portulaca* would be occupied by *P. umbraticola*. A lot of new *P. umbraticola* cultivars have been recently bred with different flower color, flower diameter and flower longevity. Most of these characteristics associated with the new cultivars have not yet been fully examined, here we report the examination of some of the cultivars with an aim of further developing the breeding of ornamental *Portulaca.*

Determination of Nuclear DNA Content and Ploidy Level Estimation

Nuclear DNA content and ploidy level estimation of 29 *Portulaca umbraticola* cultivars was done by flow cytometry analysis using *Ipomoea nil* ‘Violet’ as an internal standard. The nuclear DNA content of *Portulaca oleracea* was also estimated using the same method. The materials were placed

into three distinct groups a, b, and c based on the Relative Florescence Intensity (RFI; Specimen/*I. nil*). Three ploidy level groups were estimated, 2x, 3x and 4x thus a, b and c, respectively. The nuclear DNA content of the 2x, 3x, and 4x *P. umbraticola* cultivars was two, three and four folds that of the internal standard, respectively. Although *P. oleracea* is 6x, its nuclear DNA content was very low, less than that of 2x *P. umbraticola* cultivars thus *P. oleracea* has a smaller genome size than *P. umbraticola* cultivars. Authenticity of the specimens was done through examination of the seed surface structure using a digital microscope, also all *P. umbraticola* cultivars consists of the wing-pod, a typical characteristic used for identification of these species. The chromosome numbers of some of the specimens from group (a) were reported by Shibata (1991), as 18. *P. oleracea* have 54 chromosomes. Assuming the base chromosome number x = 9, the estimated ploidy levels proved to be correct.

Difference in Flower Longevity and Endogenous Ethylene Production

Portulaca umbraticola Kunth, with ephemeral flowers, has become an important summer bedding plant in Japan. A lot of new cultivars have recently been bred with different flowering characteristics, but there is little information about *P. umbraticola* cultivars. In this study, we investigated the differences in flower longevity, endogenous ethylene production and ethylene sensitivity between a conventional cultivar, 'Single Red' (SR), and a newly released cultivar, 'Sanchuraka Cherry Red' (SCR). The flowers of SR opened and closed earlier than those of SCR and the flower longevity of SCR was significantly longer than that of SR. The effects of pollination, filament wounding and pistil removal on flower longevity were also investigated in both cultivars. Pollination, filament wounding and pistil removal significantly accelerated senescence in both cultivars, but filament wounding was much more significant in accelerating senescence. Endogenous ethylene production from flower opening to closure was significantly higher in SR than in SCR. The peak ethylene production in SR occurred 2 h earlier than that in SCR. Exogenous ethylene treatments of 0.5, 1, and 2 $\mu L \cdot L^{-1}$ significantly accelerated the rate of senescence in both SR and SCR. The use of ethylene action inhibitor 1methylcyclopropene (1-MCP) and ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) significantly improved flower longevity in both cultivars, with the latter being much more effective. The better flower longevity of SCR seems to be related to lower

endogenous ethylene production. The senescence of *P. umbraticola* cultivars seems to be ethylene-dependent.

Effect of Different Photoperiods on Flower Opening Time in *Portulaca umbraticola*

Portulaca umbraticola is an ephemeral flower that opens early in the morning and wilts in the late afternoon. Although light and temperature act as major external cues to limit the velocity of flower opening, endogenous factors regulating its timing are largely unknown. In this study, we used time lapse photography to study the effect of different photoperiods and light qualities on the flower opening rhythm of *P. umbraticola*. When illumination was provided, flower opening was rapid and most of the flowers reached the full opening stage. In contrast, in continuous darkness (DD), progression of flower opening was similar to other treatments only during the earlier stages of flower opening; thereafter, progression was significantly slower and most flowers did not progress up to the full opening stage. A robust flower opening rhythm with a period of approximately 24 h was observed in DD for at least three days and flower opening was strongly synchronous. In contrast, continuous white (LL) and continuous red (RR) lights showed a less robust rhythm with periods of approximately 21 and 22 h, respectively, for the first two days and from the second to the third day arrhythmia developed. Continuous blue light (BB) mirrored DD, with a period of approximately 25 h. Under the different photoperiods used (20L/4D, 18L/6D, 16L/8D, 12L/12D, 8L/16D, and 4L/20D), flower opening occurred earlier at longer photoperiods in comparison with shorter photoperiods, relative to the reference point (17:00). However, when the dark period was less than 6 h, loss of synchronicity of flower opening was observed. Synchronicity of flower opening was only set when the dark period was greater than or equal to 6 h.

The new cultivars with larger flower diameter and better flower longevity might be of different polyploidy level compared to the conventional cultivars. The degree of polyploidy had been strongly correlated to organ size in most plant species. The flower of *P. umbraticola* is ephemeral and generally opens early in the morning and wilts in the afternoon, the flowers do not fully open on a cloudy or relatively cool days. In 2012 a new series (Sanchuraka) of *P.umbraticola* cultivars with better flower longevity was released. In this report, variation of polyploidy level and flowering characteristics among *P.umbraticola* cultivars was investigated.

***De novo* Transcriptome Analysis of *Portulaca umbraticola* by RNA-seq**

Here, I report the first comprehensive transcriptome information of *Portulaca umbraticola*, with a bias towards the discovery of ethylene biosynthesis, ethylene perception, and ethylene signalling genes as well as the plant circadian clock related genes. I generated 22.15 Gb bases which were assembled into 68, 928 unigenes having a total length, average length, N50 and GC content of 68, 287, 771 bp, 990 bp, 1621 bp, and 42, 78 %, respectively. The unigenes were annotated with 7 functional databases, and from the results I detected 40, 712 CDS, additional 3, 168 CDS were obtained through ESTScan. I also detected 13, 603 simple sequence reapts on 10, 784 unigenes and 1, 444 transcription factor (TF) coding unigenes were also predicted. I identified every transcript in the ethylene biosynthesis pathway, also transcripts corresponding to most core clock components were identified. Two ACS, and ACO genes were identified, and designated as *PuACS1*, *PuACS2*, *PuACO1* and *PuACO2*, respectively. Preliminary investigations on the expression profile of the ethylene biosynthesis, and ethylene perception genes were analysed in two cultivars, one with long vase life and another with a short vase life. P*uACS1* and *PuACO1* transcripts were high, and peaked earlier in short lived cultivar than long lived cultivar consistent with the observed endogenous ethylene production. Transcripts of the receptor genes were also high in short lived cultivars than long lived cultivars, *PuETR2* seems to have been upregulated by ethylene. As for the core clock components, preliminary investigations have shown conservation of clock function in *P. umbraticola* with some small divergences also observed. *PuCCA1*, *LHY* peaked at dawn, during their peak time *TOC1* transcripts were at trough levels and as dusk *CCA1* and *LHY* transcripts starts decreasing reaching trough levels at dusk, at this point *TOC1* reached its peak. These robust rhythms were maintained in continuous darkness confirming the transcripts expression is under control of the circadian clock.