BioResource Now !

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Discovery of a Protein that Enhances Flower Pigmentation Using a Novel Mutation in Japanese Morning Glory

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Many mutations exist in the Japanese morning glory. Some of these occurred in the late Edo Period (between 1600 and 1868), when jumping genes or transposons in the *Tpn1* family were inserted into other genes. Approximately 800 transposons exist in the genome of the Japanese morning glory, and these transposons still possess the jumping activity. Therefore, frequently, a novel mutation occurs. By using one such novel mutation, a protein that enhances flower pigmentation was discovered.

Research Background

In many flowers, coloration is by the pigment anthocyanin. Anthocyanin is a typical pigment in plants, and is a type of polyphenol called a flavonoid. The depth of flower color is determined by the amount of anthocyanin contained in the flower; the color is deeper when there is more anthocyanin. Previously, the factors and mechanisms that control the amount of anthocyanin were not sufficiently known.

Appearance of a Mutation that Makes Flower Color Paler

This study used a new mutation that reduced the anthocyanin content, thus, making flower color pale. This mutation was not artificially produced but naturally appeared from a commercially available strain called Benichidori. The mutation did not directly appear from Benichidori, however, but rather appeared after three mutations had repeatedly occurred (Fig. 1).

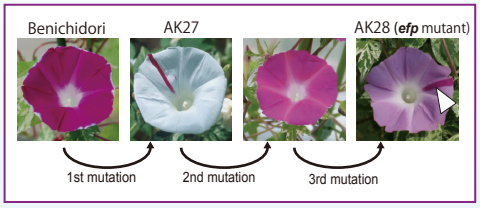

Fig. 1: Dark red spots (indicated by the white arrow) are observed on a light pink flower of AK28.

The first mutation changed Benichidori flowers from dark red to having red spots on a white background. This mutation was discovered by a layperson in Toyama Prefecture in 2000. We obtained the seeds of this strain and cultivated them. Subsequently, two mutations occurred. One was that the mutation that had occurred in Toyama Prefecture was reversed to have the original red flower. The other, which made flower color paler, was used in this study. This strain (AK28) with light pink flowers, which was obtained by a series of lucky accidents, was collected by the National BioResource Project (NBRP) Morning Glory as a new resource.

Discovery of an Enhancer of Flavonoid Production

On a light pink flower of AK28, spots in original dark red of Benichidori are observed. When the seeds of AK28 are sown, a reverse mutant whose flower is in dark red, the same as that of Benichidori, can be obtained. This phenotype is often observed in a mutation in which a transposon participates. On the assumption that a transposon in the *Tpn1* family of Japanese morning glory had mutated, we searched for the causative gene. In the search, the transposon display method was used, in which a DNA sequence that connects the transposon is amplified using polymerase chain reaction (PCR) (Fig. 2). The principle of this method is that since a transposon is inserted into a mutant, DNA fragments derived from the causative gene can be amplified in the mutant, but the DNA fragments cannot be amplified in wild-type plants or reverse mutants. The causative gene found in this method encoded a protein whose function had been unknown.

We investigated the flower pigments and found that the protein facilitated the biosynthesis of not only anthocyanin but also a flavonoid that is a colorless flavonol. Therefore, we named this protein "enhancer of flavonoid production" (EFP). EFP can triple the efficiency of anthocyanin production.

We further investigated the *EFP* gene in 39 strains with light-colored flowers collected by the NBRP Morning Glory. Of these, 32 possessed the loss-of-function *EFP* gene. Similar to Japanese morning glory, EFP in *Petunia sp*. and *Torenia fournieri* was revealed to increase the efficiency of anthocyanin production.

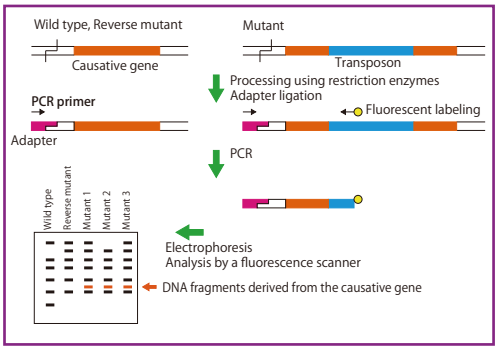

Fig. 2: Transposon display method.
In a mutant, a transposon is inserted into the causative gene. The genomic DNA is degraded by restriction enzymes, each fragment is connected with an adopter, and PCR is performed using primers for the adopter and the transposon. Since fluorescent labeling has been applied to the primer for the transposon, only DNA fragments amplified by the primer for the transposon can be detected when the electrophoresis images are analyzed using a fluorescence scanner (DNA fragments amplified by the primer for the adapter cannot be detected). DNA fragments that are derived from the causative gene and commonly appear in mutants are collected from the gel, the base sequence is determined, and finally, the causative gene is identified.

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Future Perspective

At present, the mechanism by which EFP increases the efficiency of anthocyanin production is still unknown. We believe that the elucidation of this mechanism will lead us to understand the mechanism for increasing the efficiency of matter production in plants. Similar to the present study, we will be able to find a new mutation and its causative gene using transposons in Japanese morning glory. We have been screening new mutants using transposons and expect to obtain mutants with higher scientific and aesthetic values than ever.

Reference

Morita, Y, Takagi, K, Fukuchi-Mizutani, M, Ishiguro, K, Tanaka, Y, Nitasaka, E, Nakayama, M, Saito, N, Kagami, T, Hoshino, A, Iida, S. (2014) A chalcone isomerase-like protein enhances flavonoid production and flower pigmentation. *The Plant Journal* 78, 294-304. Doi:10.1111/tpj.12469

Registering links for the LinkOut service at PubMed

Ongoing Column [No. 89] 10 min

The NCBI database offers a service called “LinkOut” that allows you to register links to related websites. This service is commonly used to provide a link from PubMed to the free full text version of a publication. Moreover, the service allows users to easily check whether a university library subscribes to the publication.
We have recently registered NBRP as a LinkOut resource provider, allowing users to access the NBRP website and make requests directly from publications that utilize NBRP resources. This article provides an overview of the registration process.

Fig.1 shows the Abstract page from PubMed. The LinkOut section is highlighted in red.

NBRP has established the Resource Research Circulation (RRC) service as a means for receiving feedback from users. RRC is a website for registering the metadata of publications that utilize NBRP resources, and more than 12,000 items have been registered already. We have thus decided to provide this data on PubMed's LinkOut.

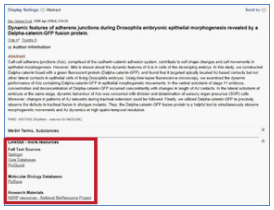


Fig. 1. The Abstract page at PubMed

① You must first send an email to NCBI requesting to register as a provider. Include the following information in the body of the email, and send it to NCBI (linkout@ncbi.nlm.nih.gov) in English:

- Organization name
- Name of the person in charge
- Email address
- Telephone number
- Postal address
- URL of the link destination
- Database and the scope for linking

We contacted NCBI to supply them with an explanation of NBRP, including information on RRC, and we have asked for links to be created for publications that utilize resources provided by NBRP. We received a reply within two days—and after exchanging two or three further emails and answering a question regarding the volume of data—we were asked to submit sample files.

② Submit the following two XML files as samples

■ providerinfo.xml※1

This file contains information about the provider.
The provider number is assigned at the time of registration.

Example provider information XML file for RRC

```
<?xml version="1.0"?>
<!DOCTYPE Provider PUBLIC "-//NLM//DTD LinkOut 1.0//EN"
"http://www.ncbi.nlm.nih.gov/projects/linkout/doc/LinkOut.dtd">
<Provider>
  <ProviderId> Provider ID </ProviderId>
  <Name> National BioResource Project</Name>          <!-- Organization Name -->
  <NameAbbr>jpnbioresp</NameAbbr>                     <!-- Abbreviation -->
  <SubjectType>culture/stock collections</SubjectType>  <!-- Category -->
  <Url> http://rrc.nbrp.jp/</Url>                     <!-- URL -->
  <Brief>The database of papers related to the NBRP resources.</Brief>  <!--Brief overview -->
</Provider>
```

■ Resource File※2

This file contains information required for the link.
The process is especially simple if there is only one target link

For a publication with a PubMed ID of 7549, the LinkOut link created based on the XML file in the above example would be as follows:

http://rrc.nbrp.jp/referenceListAction.do?pmid=7549

You can create configurations that are more complex, or you can include other publication metadata (ISSN, PII, and DOI codes) using URL parameters.

Example resource file XML file for RRC

```
<?xml version="1.0"?>
<!DOCTYPE LinkSet PUBLIC "-//NLM//DTD LinkOut 1.0//EN"
"http://www.ncbi.nlm.nih.gov/projects/linkout/doc/LinkOut.dtd"
[<!ENTITY base.url
"http://rrc.nbrp.jp/referenceListAction.do?">]>
<LinkSet>
 <Link>
  <LinkId>1</LinkId>
  <ProviderId> Provider ID </ProviderId>
  <ObjectSelector>
   <Database>PubMed</Database>                    <!-- Source database from which the link is created -->
   <ObjectList>
    <ObjId>7549</ObjId>                           <!-- PubMed ID of the first article for linking -->
    <ObjId>15983</ObjId>                          <!-- PubMed ID of the second article -->
   <ObjId>22535</ObjId>                           <!-- PubMed ID of the third article -->
   <ObjId>35528</ObjId>                           <!-- PubMed ID of the fourth article -->
    ...
   <ObjId>24843139</ObjId>                        <!-- PubMed ID of the 100th article -->
   </ObjectList>
  </ObjectSelector>
  <ObjectUrl>
   <Base>&base.url;</Base>                        <!-- The base URL specified at the beginning of the document -->
   <Rule>pmid=&lid;</Rule>                        <!-- Link parameter -->
   <UrlName>NBRP resources</UrlName>              <!-- Title used in the link -->
  </ObjectUrl>
 </Link>
```

※1 Help page for providerinfo.xml
http://www.ncbi.nlm.nih.gov/books/NBK3807/#files.Identity_File

※2 Help page for the Resource File
http://www.ncbi.nlm.nih.gov/books/NBK3807/#files.Resource_File

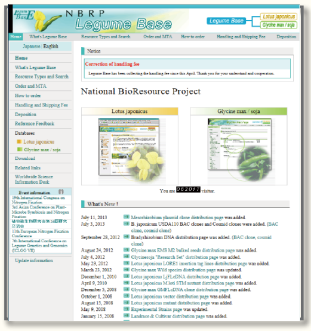
Webpage for validating created XML files
http://www.ncbi.nlm.nih.gov/projects/linkout/doc/validate.shtml

Given that the submitted files are appropriate and valid, an FTP account will be created for you at a later date. After uploading the required files via FTP, the registered links will be displayed on PubMed within 48 hours. You can update links using the same method. A monthly access report for registered links will be automatically sent to you. This convenient report includes the journal name and the number of times the link was accessed.
Although I used publications as an example in the article, you can also use the LinkOut functionality for various databases published by NCBI.

(Hiroki Watanabe)

Database of this Month

National BioResource Project “Legume Base”
(*Lotus japonicus, Glycine max/soja*)



Number of strains:9,354
Number of DNA clones:239,164
(As of June 2014)

DB name：Legume Base
URL ：http://www.legumebase.brc.miyazaki-u.ac.jp/
Languages：Japanese, English
NBRP Legume Base：
Lotus japonicus and *Glycine max* resources can be ordered from this website.
Lotus japonicus (Database of *Lotus japonicus* resources): Experimental strains, wild accessions, RI lines, EMS mutant lines, *Mesorhizobium loti* STM mutants, and DNA clones (BAC, TAC, and cDNA)
Glycine max/soja (Database of *Glycine max/soja* resources): Cultivars, wild accessions, RI lines, RI (MxS) lines, Mutants, Edamame, and full-length cDNA
Features:
- Wild accessions can be searched using the place of collection, weather conditions, and phenotype (*Lotus japonicus*).
- A research set of wild accessions is available in which all places of collection are included (*Glycine max/soja*).
- General information about experimental strains is available (*Lotus japonicus*).
- All resources can be ordered from the website.

Cooperative DB: *Lotus japonicus* EST index (Kazusa DNA Research Institute), Miyakogusa.jp (Kazusa DNA Research Institute), rsoy (Riken Plant Science Center)
DB construction group: NBRP Legume Base, NBRP Information
Management organization: Miyazaki University
Year of first DB publication：2004 Year of last DB update：2014

Comment from a developer: This database is composed of three sub-databases—the NBRP Legume Base, *Lotus japonicus* Database, and *Glycine max/soja* Database. The NBRP Legume Base provides resources concerning *Lotus japonicus* and *Glycine max*. The *Lotus japonicus* Database is in charge of providing information about *Lotus japonicus* resources. The *Glycine max/soja* Database is in charge of providing information about *Glycine max* resources. These three sub-databases are cooperatively managed. The system adopted by this database was established due to historical circumstances. These sub-databases have different functions, so functions can easily be added. Therefore, many new resources can be exhibited within a short period, and users can quickly use them. Ten years have passed since the first publication of the NBRP Legume Base. During this period, we have managed this database without major changes. At present, we are preparing a new design for this website so that the new database will be easier to use than ever.

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Editor's Note

The Japanese morning glory freshly blooms in summer mornings and is very attractive. Not only devotees but also regular people want to grow several types of Japanese morning glory every year. We are fascinated by the various flower colors; Assistant Professor Atsushi Hoshino and his group discovered a gene that controls their color intensity. Since the function of its causative gene was not known, this discovery has attracted the attention of researchers interested in its application. I look forward with pleasure to further developments (Y. Y.).

BioResource Information

(NBRP) www.nbrp.jp/
(SHIGEN) www.shigen.nig.ac.jp/
(WGR) www.shigen.nig.ac.jp/wgr/
(JGR) www.shigen.nig.ac.jp/wgr/jgr/jgrUrlList.jsp

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