

Enhancement of Characterization and Variety Identification Technology in the Plant Variety Protection System

Assessment of type of bearing for Strawberry Varieties by using DNA marker

Yoshiyuki Ohno

Plant Variety Protection Office, Intellectual Property Division
Export and International Affairs Bureau, MAFF of Japan



Background and objective

Four-season bearing (day neutral) varieties have a longer flowering and maturing period than one-season bearing (no remontant) varieties. Breeding activities four-season bearing (day neutral) varieties in Japan has not long history and has a potential to develop excellent varieties will be expected near the future.

In the DUS growing test, to assess the type of bearing, the plant should cultivate under the natural low temperature condition in outdoor field in winter, and it needs to continue cultivation to summer to check the presence of flowering under the lighting cultivation.

[Existing matters to be resolved as follows]

- It cannot be assessed by heating facility(greenhouse) which is the main cultivation type of one-season (no remontant) varieties in Japan.
- Even if outdoor cultivation type, there is a matter for extension of duration of cultivation additional 6 months required just for assessing type of bearing.

In this project, it will assess the practicality of a simple and rational method for assessing type of bearing using the suitable DNA markers.

Schedule for current DUS test at Center for Seeds and Seedlings, NARO (NCSS)



November	December to March	End of April	July to October
Planting	Meet low temperature	Finish fruit assessment	Assessment to the type of bearing under the lighting cultivation which needed additional 6 month.

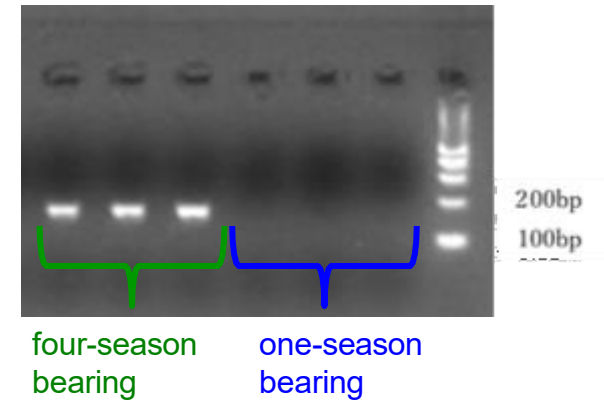
A red arrow points from the start of the 'Assessment to the type of bearing' phase in July to the end of the 'Finish fruit assessment' phase in April, indicating a 6-month extension. A blue arrow points from the start of the 'Meet low temperature' phase in December to the end of the 'Finish fruit assessment' phase in April, indicating the current testing period.

The aim of project is shortening testing period by using DNA markers.

Assessment Four-season bearing by DNA marker

1. DNA marker with 150bp (agarose gel) [Gel electrophoresis]

- The simplified marker for assessing.
- Specific amplified DNA fragments linked to the four-season gene (*Evb*) (about 150 bp)
- It may not be consisted assessment result with the phenotype(89% concordance rate). (Horticulture J. 89: p161-, 2020)



2. DNA marker with 151bp (SSR) [Analysis by Sequencer]

- Most closed marker to the *Evb*.
- 151 bp* amplified DNA fragment linked with *Evb*.
- Four-season varieties: All varieties have 151 bp fragments
- One-season varieties: Normally don't have 151 bp fragments however some varieties have 151 bp fragments with unlinked *Evb*. (Euphytica 209: p293-, 2016)

DNA marker with 150bp (agarose gel)

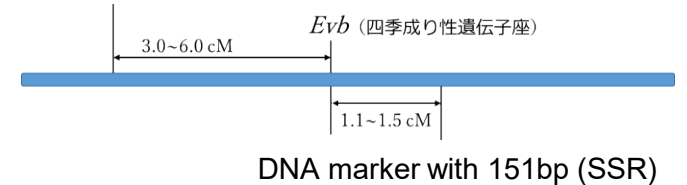
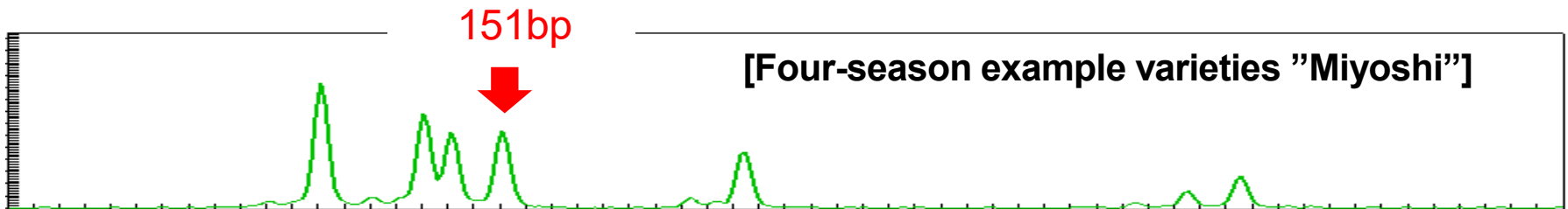
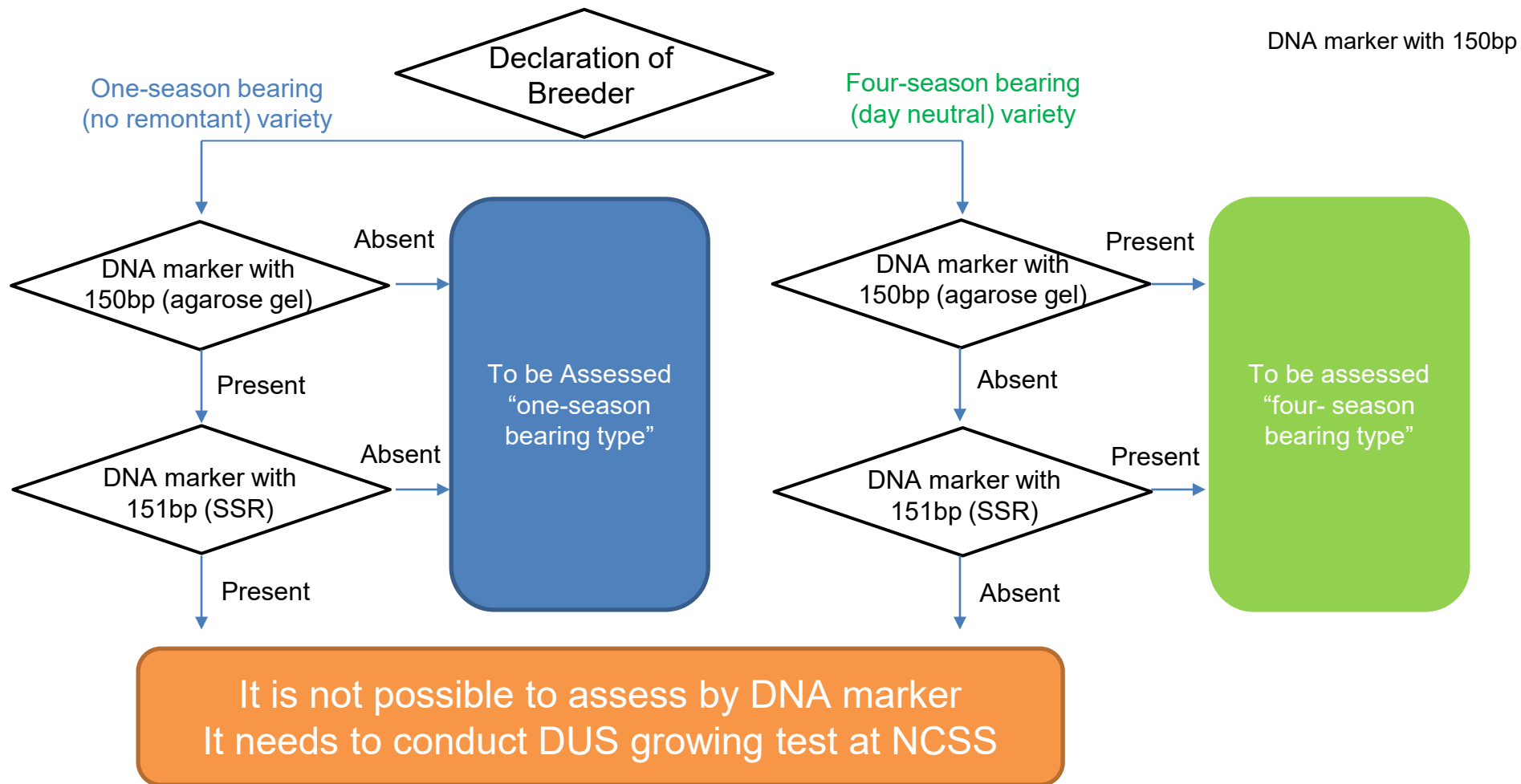


Image of relation to the linkage of two markers



Draft flowchart of assessment type of bearing by using DNA marker (under the consideration)



- **Type of bearing could be assessed when the breeder's declaration and the analyze result of one of the two DNA markers matched.**
- **If it is difficult to assess by DNA marker, type of bearing should be conducted DUS test.**

Future Challenges

- The assessment is based on the consistency with the type of bearing declared in the TQ from the breeder. It is important to declaration from the breeder should be suitable as possible. In the case of four-season bearing varieties, the photographs of flowering and maturing fruits in the summer is desirable that it would be attached to the application form.
- This assessment method cannot be completely assessed by using only DNA marker yet. In the future, the accuracy of DNA markers to be expected to improve.
- Note that this marker has been patented. Permission is required from the right holder when using this marker.



Thank you very much for your attention!