RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 56 Issue 2: 214-219 (April 2022)

Application of a Visible/Near-infrared Spectrometer in Identifying Flower and Non-flower Buds on 'Fuji' Apple Trees

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10.18805/IJARe.A-655

ABSTRACT

Background: Forecasting bud physiologic conditions can help 'Fuji' apple farmers manage their orchards more efficiently. Being able to determine the nature of a bud before bud burst is one such forecast that could be of use to these 'Fuji' growers. The aim of this research project was to determine if a device, a visible/near-infrared spectrometer, could be employed to determine whether a bud is a flower or non-flower bud without destroying the bud.

Methods: Experiments were conducted on buds taken from a 'Fuji' apple tree, beginning on January 29 through to March 31, 2021, three days before bud burst. The data from the visible/near-infrared spectrometer clarified whether a bud was a flower or a non-flower bud. The Spectro data Classification Learner App proved to be an accurate classification method to analyze flower and non-flower bud Spectro data.

Result: Three days before bud burst, the chlorophyll content levels of the non-flower buds were markedly higher ($P \leq 0.05$) than those of the flower bud, which explains why the visible border of the near-infrared spectrometer might have been changed by the chlorophyll content of buds. The visible and near-infrared bands of the buds showed that the Spectro data of the non-flower buds were higher than those of the flower buds when measurements were made three days before bud burst. Three days before bud burst Cubic KNN of KNN classifier analyzed flower and non-flower buds smoothly. Spectro data were labeled as accuracy 75.9%, sensitivity 86% and specificity 67%. The results that were obtained suggest that farmers could use a visible/near-infrared spectrometer to identify flower and non-flower buds in their orchards, without damaging the buds, three days before bud burst.

Key words: Bud burst, Chlorophyll content, Classifier learner app, Non-destructive method.

INTRODUCTION

The 'Fuji' apple (*Malus domestica* Borkh.), a cross between 'Delicious' and 'Ralls Janet,' was introduced in Japan in 1962 (Soejima *et al*., 1998). This fine-grained apple, with its high sugar and low acid content, is juicy, firm and crisp and has a sweet, spicy flavor (Rojas-Grau *et al*., 2006). Today, it is one of the world's most widely consumed apples and is cultivated in apple-producing regions across the globe.

On the other hand, the 'Fuji' poses a number of problems for growers. Due to its vigorous growth, it is necessary to prune aggressively in order to open up the canopy to control this growth. In the case of the 'Fuji,' it is crucial to distinguish between flower and non-flower buds when pruning, because if these buds are not identified and flower buds thinned, they will cause over-vigorous growth and lower productivity.

Chlorophyll is the pigment that gives a plant its green color and is a crucial component of a plant's physiology (Palta, 1990; Gitelson *et al*., 2003). Until now, the bud chlorophyll content of 'Fuji' apple buds has not been used to identify flower and non-flower buds. Moreover, no research has been reported on the detection of flower and non-flower buds using non-destructive measurement methods. Therefore, we decided to check bud chlorophyll content and changes in chlorophyll levels, and to use a visible/near-infrared spectrometer to identify and enable the separation of flower from non-flower buds. In this study, the classification of flower and non-flower buds was determined by a near-infrared and visible border that identifies chlorophyll content. However, although identifying changing levels of chlorophyll in the weeks/days leading up to bud burst can determine which buds are flower buds and which are non-flower buds it also involves the destruction of the bud itself. On the other hand, it offers verification of and insights into non-destructive approaches.

Buban and Faust (1982) have reported that determining whether a bud is a flower bud or non-flower bud is crucial for 'applied horticulture' and the future productivity of a young orchard. Flower bud formation is a complicated process because it is affected by the tree's spurs and long shoots, the character of the cultivar, as well as the age and strength

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How to cite this article: Botirov, A., An, S., Arakawa, O. and Zhang, S. (2022). Application of a Visible/Near-infrared Spectrometer in Identifying Flower and Non-flower Buds on 'Fuji' Apple Trees. Indian Journal of Agricultural Research. 56(2): 214-219. DOI: 10.18805/IJARe.A-655.

Submitted: 18-05-2021 **Accepted:** 04-09-2021 **Online:** 22-09-2021

of the tree (Pratt, 1988). Moreover, apple tree flower bud growth emerges on different parts of the tree. However, smart agriculture technologies now make it possible to discern bud characteristics before pruning, without destroying the bud in the process. The device that can be used to determine hidden parts of examined objects is the visible/near-infrared spectrometer. This spectrometer is easy to use and produces results quickly.

Non-destructive testing includes a broad range of techniques that are used in science and technology industries to evaluate a material, component, or a system's properties without causing damage. According to Crowley (2020), the visible region of the spectrum of electromagnetic radiation identified by a visible/near-infrared spectrometer is typically considered to be made up of wavelengths ranging from 400 nm (violet light) to between 700 and 800 nm (red light). Manley and Baeten (2018) have noted that, "the essential origins of near-infrared spectroscopy include the production, reporting, and understanding of spectra resulting from the interaction of electromagnetic radiation with an object." Corresponding to Gogoi *et al.* (2018) spectroscopical and photographical equipment had been used for discovering plant disorders and Venkatesan *et al*. (2020) near-infrared spectrometer had been used for crops seed characteristics. Osborne (2000) reported that "the infrared (IR) region comprises that part of the electromagnetic spectrum in the wavelength range between 780 and 100,000 nm and is divided into near-IR, mid-IR and far-IR subregions; the near-infrared region covers the wavelength range from 780 to 2500 nm." Furthermore, Rathore *et al*. (2021) citated that near-infrared spectrometer include from 700 nm to 2500 nm wavelength ranges. Therefore, we use a visible/near-infrared spectrometer to identify and enable the separation of flower from non-flower buds.

The aim of this research project was to detect flower and non-flower buds on 'Fuji' apple trees before bud burst without destroying the buds. To do so, we 1) analyzed buds before bud burst using an ultra-mini visible/near-infrared spectrometer and 2) measured the chlorophyll content before bud burst to explain what was visible on the spectrometer. Results showed that the most reliable spectrometer readings of flower and non-flower buds occurred three days before bud burst.

MATERIALS AND METHODS

Plant materials

Flower and non-flower buds from a 'Fuji' apple tree were used in this study. The 'Fuji' tree studied was in one of the orchards located on the grounds of the "Hirosaki University Fujisaki Research Station" (Fujisaki, Aomori Prefecture, Japan). The tree used was ten years old and had been grafted on semi-vigorous Marubakaido rootstock. The dates selected to test the buds were January 29, February 15, March 1, March 15 and March 31 (accordingly 64, 47, 33, 19 and 3 days before bud burst), the latter being three days before the tree's 2021 bud burst (Table 1). Buds used for testing on the respective testing dates, different branches were taken randomly from the same tree. On each of these dates a branch was cut off and brought to a laboratory in the Hirosaki University Faculty of Agriculture and Life Science. There, the buds were separated from the branch and examined with an ultra-mini visible/near-infrared spectrometer and tested for chlorophyll content as well.

Non-destructive measurement

The visible/near-infrared spectrometer is a device that measures the amount of light that passes through an object without destroying it. The OMT-NIR-M1 spectrometer used in this study was manufactured by Optcom Co., Ltd. using SpectralRatio Version 1.1.0.1 software. This spectrometer measures from a range of 640 nm to 1050 nm, with an interval of 2 nm. Measurement parameters were adjusted to amp gain-high, to memory integration-16 and to smoothing points-16 nm. All buds were measured with this spectrometer and the spectral data were collected. The buds were then examined under a microscope and the spectral data were used to determine whether a bud was a flower or a non-flower bud.

Grouping into flower and non-flower buds

The buds were weighed and sliced in two with a razor blade from the middle of the top through to the bottom of the bud and then checked with an Olympus microscope (Olympus Corporation Tokyo, Japan, made in the Philippines). In the upper part of the flower buds was a yellowish-green oval stamen, whereas in the upper part of the non-flower buds there was no such oval, though there was some light green matter (Fig 1 A and B). The flower buds were then separated from the non-flower buds and their chlorophyll content was measured.

Measurement by destructive means

The flower and non-flower buds were classified by shape, weight, and also by chlorophyll content. Bud chlorophyll content is related to bud physiology and differs depending upon the type of bud. The separated buds were carefully placed inside 2 ml tubes and pulverized in a Homogenizer ShakeMan6 (model PS-SMNO6), after which the buds were infused for 10 minutes in a 1.5 ml 80% acetone solution inside 2 ml tubes. The mixtures were then moved to different 1.5 tubes, which were then put into a high-speed Micro Centrifuge and set at a speed of 140,000 (rpm), for 10 minutes. The liquid rose to the top of the tubes and debris from the buds settled at the bottom. The liquid solution at the top of the tubes was then moved into a quartz glass

Table 1: Flower and non-flower buds: destructive and non-destructive testing and their distribution until bud burst for 'Fuji' in January through March in 2021.

Name	Number of buds			
	29-Jan	1-Mar	15-Mar	31-Mar
Flower	17	23	20	17
Non-flower	7	10	9	12
Total bud	24	33	29	29

Bud burst occurred on April 2, 2021.

tube in order to measure the chlorophyll content with a ultraviolet-visible (UV) spectrophotometer (Shimadzu Access Corporation). The UV spectrophotometer was set at three measuring ranges (645, 663 and 750 nm) and chlorophyll content was checked within these Spectral ranges and calculated using the following equation (1):

$$C = 7.22\ (A663\ nm - A750\ nm) + 20.30\ (A645\ nm - A750\ nm) \quad ......(1)$$

Statistical analysis

Chlorophyll levels were analyzed using a one-way ANOVA (comparing the difference between dates) and a Tukey test. Flower and non-flower bud chlorophyll content differences were analyzed using the Student's t-test. All of the above analyses were conducted by applying the R studio version 1.3.1073 (© 2009-2020 RStudio, PBC). Different software was used to obtain Spectro data.

The bud Spectro data were analyzed using MATLAB R2018b version 9.5.0.1298439 (©1984-2018 the MathWorks, Inc), with the Classification Learner App tool. Spectro data were analyzed individually for the dates on which the measurements were taken and each new session was set as cross-validation folds: 10 without PCA. Although all 22 machine-learning algorithms in the Classification Learner App were chosen, we used only the results of 9 of these in our table (Table 2). Accuracy, sensitivity and specificity were verified by a confusion matrix plot.

RESULTS AND DISCUSSION

Changes in bud chlorophyll content

Flower and non-flower bud chlorophyll levels exhibited significant differences three days before bud burst. Chlorophyll levels in the flower buds were significantly lower than those in the non-flower buds (Fig 2). Flower bud chlorophyll levels showed dramatic differences depending upon the date. These levels increased significantly with the approach of bud burst.

In this study, the classification of flower and non-flower buds was determined by a visible/near-infrared border that can identify their chlorophyll content. Until now, the bud chlorophyll content of 'Fuji' apple buds has been measured by examining the leaves and not the buds themselves. Our analysis of the buds showed that, three days before bud burst, the chlorophyll level of flower buds was significantly higher than that of non-flower buds. However, no significant differences in chlorophyll levels were found between flower and non-flower bud when measured 33 days before bud burst. This suggests that neither destructive nor non-destructive measurements of chlorophyll levels are reliable for distinguishing flower from non-flower buds.

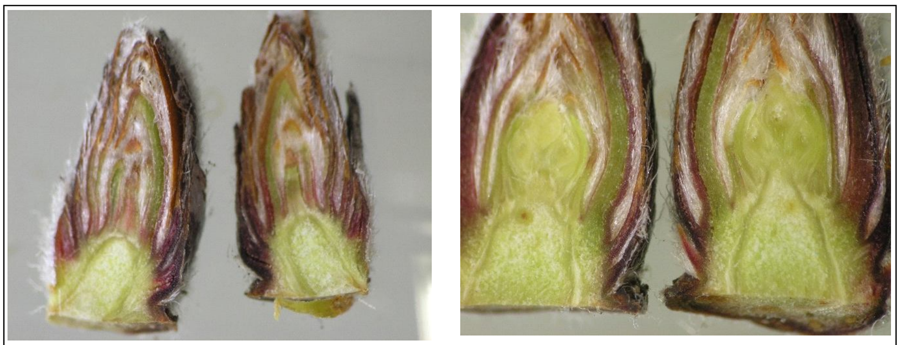
640-798 nm range

The amount of light absorbed by the non-flower buds, seen on the visible spectrometer three days before bud burst,

Table 2: Buds Spectro data classification results using the classification learner app without using the PCA three days before bud burst (DBBB) for the 'Fuji' flower and non-flower buds on the visible/near-infrared spectrometer on 2021.

Classifier	Classifier type	Classification accuracy (%) 3 DBBB		
		Accuracy	Sensitivity	Specificity
Discriminant analysis	Linear discriminant	62.1	65	56
	Quadratic discriminant	F	F	F
Logistic regression classifier	Logistic regression	58.6	65	50
KNN	Fine KNN	62.1	67	55
	Medium KNN	72.4	80	64
	Coarse KNN	58.6	59	0
	Cosine KNN	72.4	80	64
	Cubic KNN	75.9	86	67
	Weighted KNN	72.4	80	64

This table made according to analyze data by MATLAB and some of that data shown in here, F- False.



(a) (b)

Fig 1: Difference between flower and non-flower buds for 'Fuji' in January 29, 2021, (a) non-flower bud, (b) flower bud.

was higher than that of the flower buds (Fig 3). Bud Spectro absorption dropped sharply from 670 nm to 720 nm.

800-1050 nm range

The absorption in the flower bud was lower than the Spectro absorption in the non-flower bud on the as shown on the near-infrared spectrometer three days before bud burst (Fig 4). Flower and non-flower bud Spectro absorption was increased from the 930 nm and dropped on 1016 nm.

Spectro variation of buds on different dates

The flower and non-flower bud absorption seen on the visible spectrometer decreased near the approach of bud burst (Fig 5). Non-flower bud absorption observed on the visible

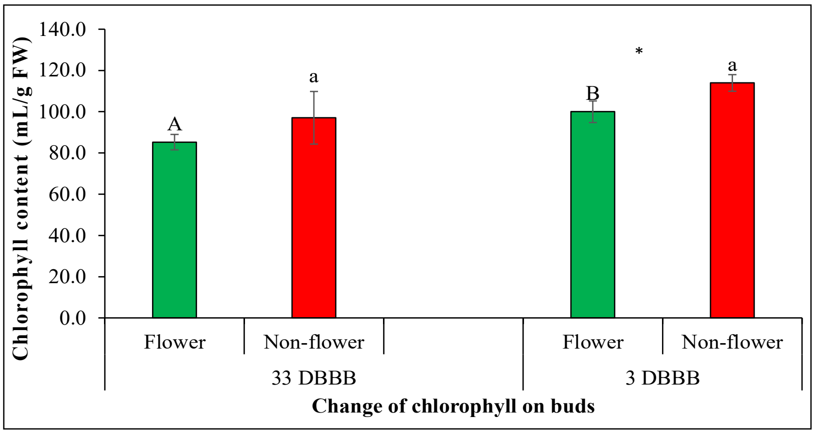


Fig 2: Changes in chlorophyll content (mL/g fresh weight): A comparison of flower and non-flower buds tested 33 and 3 days before bud burst (DBBB). Means±standard error and different letters indicate statistically significant differences between the days according to a T- test; (*)- Significant at P≤0.05.

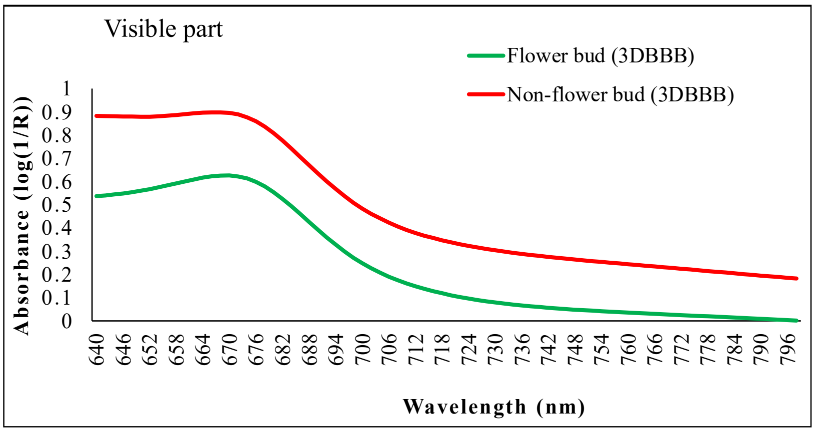


Fig 3: Flower and non-flower bud absorbance of the visible spectrometer for 'Fuji' on March 31, 2021; DBBB-days before bud burst; Green line-flower bud; Red line-non-flower bud

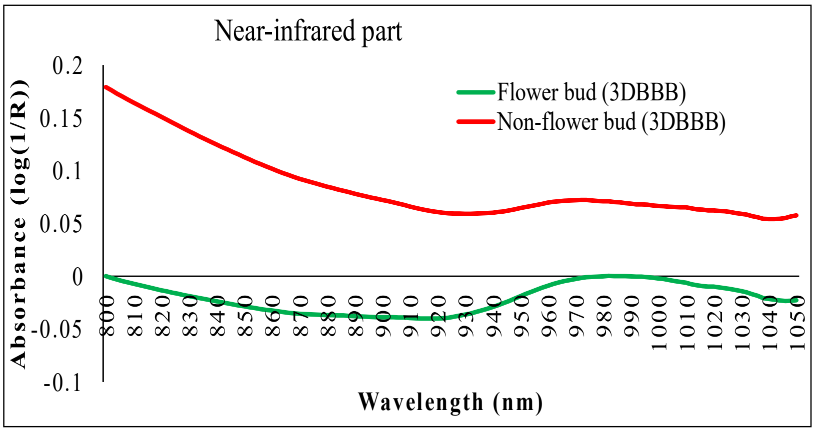


Fig 4: Flower and non-flower bud absorbance of the near-infrared spectrometer for 'Fuji' on March 31, 2021; DBBB-days before bud burst; Green line-flower bud; Red line-non-flower bud.

spectrometer 33 days before bud burst was lower than the flower bud absorption measured on the visible spectrometer. Flower bud absorption seen on the visible spectrometer was lower than that in the non-flower buds.

Absorption in the flower bud shown on the near-infrared spectrometer absorption three days before bud burst was lower than it was 33 days before bud burst (Fig 6). On the other hand, absorption in the non-flower buds seen on the near-infrared spectrometer three days before bud burst was greater than it was 33 days before bud burst. Near-infrared spectrometer light absorption readings showed that that light absorption of the non-flower buds was greater than that of the flower buds, for both 33 and three days before bud burst.

Classification analyses of spectro data

The Classification Learner App of the MATLAB was used as a classification model and the 10-fold of cross-validation was used to set the training data for the model (Table 2). The classification results were obtained using the 22 machine learning algorithms; 9 of which are also shown in Table 2. The highest classification accuracy was 75.9%, performed by cubic k-nearest neighbor (KNN), accompanied by medium KNN with 72.4% accuracy, cosine KNN (72.4% accuracy) and weighted KNN (72.4% accuracy). The highest sensitivity was found by cubic KNN (86%), then medium KNN with 80% accuracy, cosine KNN 80% (accuracy) and weighted KNN (80% accuracy).

This study introduced a non-destructive method of identifying the flower and non-flower buds of 'Fuji' apple trees. The results show that this method accurately classifies and distinguishes flower buds from non-flower buds near bud burst. This non-destructive method is an important way to ascertain chlorophyll content, the water index (Agati *et al*., 1995; Penuelas *et al*., 1997). However, this non-destructive way of differentiating the flower from the non-flower buds of 'Fuji' apple trees has once not been examined. Here, we found that the absorption of light in the flower buds shown on the visible/near-infrared spectrometer just before bud burst had diminished, whereas non-flower bud absorption had risen. Classification Learner App testing methods confirmed that the classification and differentiation of flower from non-flower buds was 75.9% accurate when tested with Cubic k-nearest neighbor (k-NN). Vitola *et al.* (2017) has reported that Cubic KNN is the simplest way to separate various data and obtain accurate results. According to Buban and Faust (1995), bud growth and development

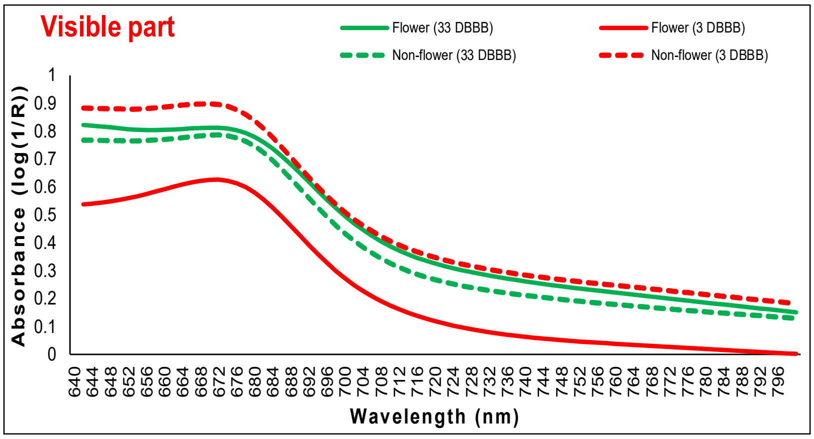


Fig 5: Flower and non-flower bud absorbance seen on the visible spectrometer for 'Fuji' on March 15 and March 31, 2021; 33 and 3 days before bud burst (DBBB); Green and red lines = Flower buds; Green and red ring lines = Non-flower buds.

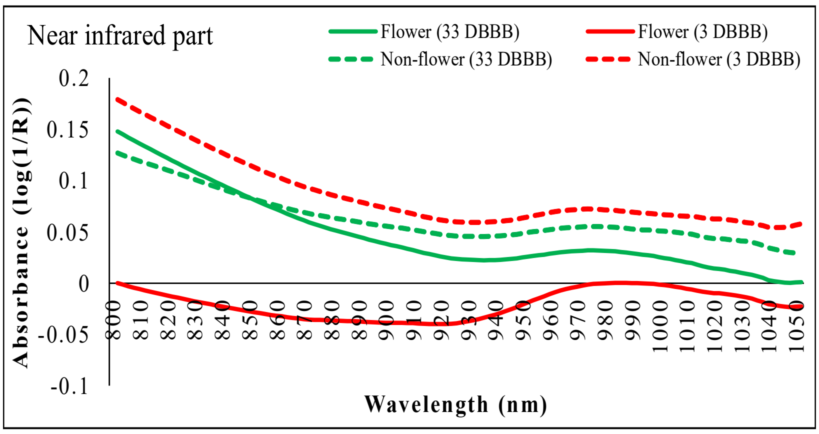


Fig 6: Flower and non-flower bud absorbance seen on the near-infrared spectrometer for 'Fuji' on March 15 and March 31, 2021; 33 and 3 days before bud burst (DBBB); Green and red lines = Flower buds; Green and red ring lines = Non-flower buds.

depend indirectly on the availability of "free water amounts of buds. Penuelas *et al*. (1997) reported that 680, 900 and 970 nm reflectance provide the best estimation of plant water content. Fruitlet drop measured by visible/near-infrared *in situ* is beneficial in terms of time efficiency and its high level of accuracy (Orlova *et al*., 2020). In previous research we reported winter planted young 'Miyabi Fuji' trunk moisture content changes (Botirov and Arakawa, 2021) and this bud light absorbance changes might be occurred some physiological changes of buds before bud burst.

Deficiency of this study is that we measured only within a restricted wavelength range (640-1050 nm). Further research should be done using a more comprehensive range on the near-infrared spectrometer (above 1050 nm).

CONCLUSION

In this study, we investigated the non-destructive detection of flower and non-flower buds before bud burst on a 'Fuji' apple tree. We found that the best time to detect and differentiate between flower and non-flower buds, utilizing a visible near-infrared spectrometer, is three days before bud burst. We also observed significant changes in bud chlorophyll in the flower and non-flower buds. This suggest that deeper non-destructive measurements especially adapted for chlorophyl might be distinguish flower from non-flower buds before bud burst.

Hence, the use of a device that does not destroy the bud can be beneficial for detecting flower and non-flower buds before bud burst and can help growers in their management of 'Fuji' apple orchards. The ultra-mini visible/near-infrared spectrometer could offer apple growers a tool that would enable them to distinguish flower from non-flower buds, which could then help them fine tune their pruning practices to better manage their orchards and forecast future harvest yields. Additionally, researchers working on smart agriculture technologies could use this data to develop pruning robots that can identify and separate flower from non-flower buds.

REFERENCES

Agati, G., Mazzinghi, P., Fusi, F., Ambrosini, I. (1995). The F685/F730 Chlorophyll fluorescence ratio as a tool in plant physiology: Response to physiological and environmental factors. Journal of Plant Physiology. 145(3): 228-238.

Botirov, A. and Arakawa, O. (2021). Root growth changes in the winter planting of young 'Miyabi Fuji' apple trees. International Journal of Horticultural Science and Technology. 8(3): 227-233.

Buban, T., Faust, M. (1982). Flower bud induction in apple trees: Internal control and differentiation. Horticultural Review. 4: 174-203.

Bubán, T., Faust, M. (1995). New aspects of bud dormancy in apple trees. Acta Horticulturae. pp. 105-112.

Crowley, T.E. (2020). Absorption of ultraviolet, visible and infrared radiation. Purification and Characterization of Secondary Metabolites. 33-48.

Gitelson, A.A., Gritz,Y., Merzlyak, M.N. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. Journal of Plant Physiology. 160(3): 271-282.

Gogoi, N.K., Deka, B. and Bora, L.C. (2018). Remote sensing and its use in detection and monitoring plant diseases: A review. Agricultural Reviews. 39: 307-313.

Manley, M., Baeten, V. (2018). Spectroscopic Technique: Near Infrared (NIR) Spectroscopy. In Modern Techniques for Food Authentication (2nd ed.).

Orlova, Y., Linker, R., Spektor, B. (2020). Forecasting the potential of apple fruitlet drop by *in-situ* Vis-NIR spectroscopy. Computers and Electronics in Agriculture. 169: 105225.

Osborne, B.G. (2000). Near-infrared spectroscopy in food analysis. Encyclopedia of Analytical Chemistry. 1-14.

Palta, J.P. (1990). Leaf chlorophyll content. Remote Sensing Reviews. 5(1): 207-213.

Penuelas, J., Pinol J., Ogaya R., Filella I. (1997). Estimation of plant water concentration by the reflectance water index WI (R900/R970). International Journal of Remote Sensing. 18(13): 2869-875.

Pratt, C. (1988). Apple flower and fruit: Morphology and anatomy. Horticultural Reviews: 273-308.

Rathore, M., Prakash, H.G. and Bala, S. (2021). Evaluation of the nutritional quality and health benefits of chickpea (*Cicer arietinum* L.) by using new technology in agriculture (Near Infra-red spectroscopy-2500). Asian Journal of Dairy and Food Research. 40(1): 123-126.

Rojas-Grau, M.A., Sobrino-Lopez, A., Tapia, M.S., Belloso, O.M. (2006). Browning inhibition in fresh-cut ' Fuji ' apple slices by natural antibrowning agents. J. Food Sci. 71: 59-65.

Soejima, J., Bessho, H., Tsuchiya, S., Komori, S., Abe, K., Kotoda, N. (1998). Breeding of Fuji apples and performance on JM rootstocks. Compact Fruit Tree. 31(1): 22-24.

Venkatesan, S., Masilamani, P., Janaki, P., Eevera, T., Sundareswaran, S. and Rajkumar, P. (2020). Role of near-infrared spectroscopy in seed quality evaluation: A review. Agricultural Reviews. 41(2): 106-115.

Vitola J., Pozo F., Tibaduiza D. A., Anaya M. (2017). A sensor data fusion system based on k-nearest neighbor pattern classification for structural health monitoring applications. Sensors (Switzerland): 17(2). 417; https://doi.org/10.3390/s17020417.