Sunlight Differentially Affects the Fruit Skin, Flesh, and Core Coloration of the Type 2 Red-fleshed Apple ‘Kurenainoyume’: Optimization of Fruit Bagging Treatment

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We investigated the effect of fruit bagging treatment using three kinds of paper bags differing in light permeability on the red coloration of skin, flesh, and core in type 2 red-fleshed ‘Kurenainoyume’ apples by comparison with type 1 ‘Goshogawara’ apples. Skin coloration of both ‘Goshogawara’ and ‘Kurenainoyume’ was affected by light and the effect was more marked in ‘Kurenainoyume’ than ‘Goshogawara’. In contrast, the effect of fruit bagging treatment on the red coloration of the core was limited in ‘Goshogawara’, while core coloration was not observed in ‘Kurenainoyume’ type 2 apples. Flesh coloration was observed even under dark conditions in both cultivars, and the light tended to enhance the flesh coloration to some extent in both cultivars. Since fruit bagging treatment is a prerequisite for ‘Kurenainoyume’ to prevent cork spot-like physiological disorder (CSPD) in the skin, we optimized the fruit bagging treatment conditions using a light impermeable double-layered paper bag (2-layer bag), considering both prevention of CSPD and the red coloration of ‘Kurenainoyume’ skin and flesh. Bag-removal at 25, 35, and 45 days before harvest (DBH), resulted in good skin and flesh coloration without CSPD incidence. Moreover, there was no significant difference in fresh weight, soluble solid, or malic acid contents compared with the non-bagging control. Therefore, we recommend bag-removal from 25 to 45 DBH for ‘Kurenainoyume’ as a practical cultivation technique.

Key Words: anthocyanin, cork spot like physiological disorder (CSPD), light, Malus × domestica, MYB transcription factor.

Introduction

The apple (Malus × domestica Borkh.), which belongs to the tribe Pyreae of the Rosaceae family, is one of the most important fruit crops in Japan, with the second highest fruit production following citrus. Apple skin color is a trait of interest, because Japanese people generally prefer red-skinned apples (Bai et al., 2016). Therefore, well-colored apples have a high market acceptance and value. However, Sugiura et al. (2013) demonstrated that poor coloration of apple skin is marked in warm regions, which could be explained by global warming; therefore, global warming threatens the apple industry in Japan. To maintain high-quality apple production with good skin coloration, Japanese growers have utilized red sport strains, such as ‘Komachifuji’ and ‘Tsugaruhime’, which are derived from ‘Fuji’ and ‘Tsugaru’, respectively. Furthermore, many practical techniques, including fruit bagging, removal of leaves, fruit rotation, and the use of reflection films, have been developed to improve skin coloration (Arakawa and Komori, 2006). Application of mixed compounds of ethephon and phosphorus-calcium have also been proposed to improve red coloration in apples (Li et al., 2002).

Red coloration in apples is the result of anthocyanin
pigments, mainly cyanidin-3-O-galactoside (Rupasinghe et al., 2010; Würdig et al., 2014). The biosynthesis of anthocyanins is mediated by multiple enzymes in the phenylpropanoid pathway, including chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose: flavonoid 3-O-galactosyltransferase (UFGT) (Honda et al., 2002; Ubi et al., 2006). It has also been shown that these genes are under the regulation of several transcription factors: MYB, bHLH, and WD40, which are involved in the regulation of anthocyanin synthesis, through the involvement of MYB-bHLH-WD40/WDR (MBW) complexes to activate anthocyanin biosynthesis-related genes (Koes et al., 2005; Ramsay and Glover, 2005). In apples, MdMYBA1/10, MdbHLH3/33, and MdTTG1 form a MBW complex for anthocyanin regulation (Espley et al., 2007; Lin-Wang et al., 2011; Pang et al., 2009; Takos et al., 2006; Xie et al., 2012); of these, MdMYBA1/10 is a key player that determines the color of apple skin (Ban et al., 2007; Espley et al., 2007; Takos et al., 2006). The functional allele of MdMYBA1/10 can generate red coloration only in fruit skin (Espley et al., 2007; Takos et al., 2006), but the R6 allele (MdMYB10R6), which has a tandem minisatellite repeat in the promoter region, leads to red skin, red flesh/core, and red leaves through autoregulation (Espley et al., 2009; Würdig et al., 2014). The apples with R6 allele are classified as type 1 (Chagné et al., 2013). Recently, in addition to skin color, several kinds of red-fleshed apples, which are defined as type 2 apples (Chagné et al., 2013), have been released in Japan and other countries including Germany, England, Switzerland, and Italy (Bars-Cortina et al., 2017; Silvestri et al., 2016), possibly due to the diversification of consumer interest. Hence, two types of red-fleshed apples (type 1 and type 2), have evolved (Würdig et al., 2014). MdMYB10R6 of type 1 apples is located in linkage group 9 (Espley et al., 2009). In contrast, MdMYB110a which controls the development of red coloration only in the fruit flesh in linkage group 17 (Chagné et al., 2013; Umemura et al., 2013). Moreover, the red-fleshed trait of type 2 apples was found to be linked to the S2-RNase allele (Sekido et al., 2010). In type 2 apples, the coloration of skin and fruit flesh is controlled by different MdMYBA1/10 and MdMYB110a genes (Umemura et al., 2013). Thus, the genetic background of red coloration has been relatively well-investigated in type 1 and 2 apples (Ban et al., 2007; Chagné et al., 2013; Espley et al., 2009; Umemura et al., 2013; Würdig et al., 2014). As for environmental factors for red coloration in apple skin have been carried out and have shown the importance of light and temperature conditions, where ultraviolet (UV) light, especially wavelengths around 310 nm, and relatively low temperature around 16°C are suitable for good coloration (Dong et al., 1995; Ubi et al., 2006).

‘Kurenainoyume’ is a new red-fleshed apple cultivar, which was released in 2010 by Hiroasaki University (Igarashi et al., 2011). ‘Kurenainoyume’ has a flat fruit shape, dark red skin like ‘Jonathan’, pink flesh, and the average fruit weight is 350 g. The soluble solid content is about 13% and the titratable acidity is about 0.9%, resulting in a sweet, mildly tart taste, that is not astringent either fresh or cooked (Matsumoto et al., 2016). Because of consumers’ interest and acceptance of this cultivar, the cultivation areas of ‘Kurenainoyume’ have rapidly expanded in the northern Tohoku region (northernmost three Prefectures of Honshu Island, Aomori, Akita, and Iwate) in Japan (Matsumoto, 2017). However, along with cultivation expansion, cork spot like physiological disorder (CSPD) in the skin has become a serious problem in recent years; the disorder markedly lowers the commercial value, resulting in a complete loss of value in the worst cases. Therefore, to meet the demand of growers, we successfully developed a cultivation technique to alleviate and/or completely prevent CSPD by fruit bagging treatment using a light impermeable double-layered paper bag (2-layer bag) from mid-July to late-August (Matsumoto et al., 2018c). This means that paper bagging treatment is a prerequisite for the production of good-quality ‘Kurenainoyume’ fruit. Contrary to skin coloration, environmental factors for flesh coloration of type 2 apple have not yet been fully investigated. Honda et al. (2017) reported that sunlight irradiation partially promoted anthocyanin accumulation in the flesh of non-bagging ‘Geneva’ (type 1) and ‘Pink Pearl’ (type 2) apples. However, considering the fact that light irradiation, especially UV light, cannot reach deep inside the fruit, it is unclear why sunlight irradiation promotes anthocyanin accumulation in the flesh of type 2 apples, albeit only slightly.

In the present study, we examined the effects of light conditions on the red coloration in apple skin, flesh, and core of type 2 red-fleshed apples compared to type 1 ‘Goshogawara’ apples to provide basic information. To this end, paper bagging treatments using a 2-layer bag were first applied to pre-harvest fruit of type 1, ‘Goshogawara’ and type 2, ‘Kurenainoyume’. Then, we used three kinds of paper bags; a 2-layer bag, a light-permeable single-layered white waxed paper bag (1-layer white bag), and a light permeable single-layered red waxed paper bag (1-layer red bag), with different levels of light permeability were applied to pre-harvest fruit to determine the sensitivity to light conditions of ‘Goshogawara’ and ‘Kurenainoyume’. After confirmation of the effects of fruit bagging treatments on apple skin and flesh coloration, we attempted to determine the optimal period for fruit bagging treatments for intense/stable red coloration in the flesh of ‘Kurenainoyume’. The results showed that light conditions differentially affected red coloration in the skin, core, and flesh de-
depending on the apple type. Our results also indicated that the flesh coloration of bagged fruit de-bagged at 25–45 days before harvest (DBH) was accelerated in ‘Kurenainoyume’. Therefore, we propose that fruit bagging treatment using a 2-layer bag, which is a prerequisite for alleviating/preventing CSPD, can also be utilized as a practical tool to improve the flesh red coloration of the type 2 apple cultivar ‘Kurenainoyume’.

Materials and Methods

Effect of fruit bagging treatment with a light impermeable 2-layer bag on the red coloration of skin, flesh, and core (Exp. 1)

The experiment was conducted in 2011 at two farms, an experimental farm of Aomori Prefectural Goshogawara Agriculture and Forestry High School, known as Gono farm (40°48’58”N, 140°29’27”E) and an experimental farm of Hirosaki University, known as Fujisaki farm (40°39’25”N, 140°29’9”E). We used two types of red-fleshed apples, type 1, ‘Goshogawara’ and type 2, ‘Kurenainoyume’; the former was cultivated at the Gono farm and latter was cultivated at the Fujisaki farm. Ten 20-year-old trees with ‘Goshogawara’ apples grafted onto M26 rootstock, trained to a leader type form (4.0 × 2.0 m planting), and five, 33-year-old (8 years after top-grafting onto ‘Jonathan’) ‘Kurenainoyume’ apple trees grafted onto Malus prunifolia rootstock, trained to a flat open center form (5.0 × 3.5 m planting) with four primary scaffolds, were used for the present experiment. Regarding the MYB allele, ‘Goshogawara’ is heterozygous of functional MdMYB10R6/no functional MdMYB1-2 (M. Hatsuayama personal communication; Takos et al., 2006); therefore, its skin, flesh, and core show red coloration due to MdMYB10R6. In contrast, ‘Kurenainoyume’ does not possess MdMYB10R6, but two functional MdMYB10R1 (=MdMYBA/I) alleles (Igarashi et al., 2011), which contribute to red skin coloration, and it is assumed that ‘Kurenainoyume’ has at least one MdMYB110a allele, considering the red flesh phenotype and the presence of the S1-RNase allele (Chagné et al., 2013; Sekido et al., 2010; Umemura et al., 2013).

For ‘Goshogawara’, approximately 10 fruitlets from each tree were randomly covered with a 2-layer bag (194 × 162 mm; Masudaya Co., Aomori, Japan) at 35 days after full bloom (DAFB) (June 21). The 2-layer bag completely prevented a light permeability from 280–750 nm (Matsumoto et al., 2018c). Half of the paper bags were removed at 96 DAFB (August 22) (bag-removal treatment), and the remaining half were covered continuously until harvest at 146 DAFB (October 11) (continuous-bagging treatment). The control fruit were not covered (non-bagging treatment). Fruit from each treatment group (n = 10) were harvested periodically from 35 to 146 DAFB, and red coloration (a* values of the Lab color space) of the skin, flesh, and core; flesh firmness; soluble solid content; and titratable acidity (malic acid content) were recorded on each sampling date.

Similar treatments were also applied to ‘Kurenainoyume’, and approximately 20 fruitlets from each tree were randomly covered with a 2-layer bag at 36 DAFB (June 22). Half of the paper bags were removed at 126 DAFB (September 20) (bag-removal treatment) and the remaining half were covered continuously until harvest at 177 DAFB (November 10) (continuous-bagging treatment). The control fruit were not covered (non-bagging treatment). Fruit from each treatment group (n = 10) were harvested periodically from 35 to 177 DAFB, and the aforementioned fruit-quality parameters were recorded.

Red coloration of the skin, flesh, and core was evaluated as a* values of the Lab color space, as multiple samples were measured simultaneously. Higher a* values indicated the strong red coloration of the sample. The a* values were measured at two points on the equatorial area using a color difference meter (NF333; Nippon Denshoku, Tokyo, Japan). After the skin was removed, the flesh firmness was measured at two points on the equatorial area of the fruit using a penetrometer with an 11.1-mm tip (FT327; Facchini srl, Alñosine, Italy). The total soluble solid content of the juice was determined using a digital refractometer (N-1; Atago, Tokyo, Japan). The total titratable acidity was measured by titration with 0.1N sodium hydroxide (NaOH) and calculated as malic acid equivalents.

Effect of fruit bagging treatment with different light permeabilities on the red coloration of skin, flesh, and core (Exp. 2)

The experiment was conducted in 2012 using the same trees as those used in Exp. 1. Three types of bags with different levels of light permeability were used to determine the effect of solar radiation intensities.

For ‘Goshogawara’, approximately 20 fruitlets from each tree were randomly covered with a 2-layer bag, a 1-layer white bag (179 × 140 mm; JA Zen-noh Tottori, Tottori, Japan), or a 1-layer red bag (195 × 173 mm; Masudaya Co.) at 44 DAFB (June 25). The one-layer white bag had 0.9–2.3% light permeability from 280–750 nm wavelength, while the 1-layer red bag had lower light permeability of 280–575 nm compared to the 1-layer white bag, but its light permeability of 576–750 nm was higher than the 1-layer white bag (Matsumoto et al., 2018b). Half of each paper bags were removed at 100 DAFB (August 20) (bag-removal treatment) and the remaining half were covered continuously until the final harvest at 139 DAFB (September 28) (continuous-bagging treatment). The control fruit were not covered. Fruit from each treatment group (n = 10) were harvested periodically from 44 to 100 DAFB, and the aforementioned fruit-quality parameters were recorded.

For ‘Kurenainoyume’, approximately 40 fruitlets
from each tree were randomly covered with a 2-layer bag, a 1-layer white bag, or a 1-layer red bag at 43 DAFB (June 25). Half of the paper bags were removed from each treatment at 129 DAFB (September 19) (bag-removal treatment), and the remaining half were covered continuously until the final harvest at 191 DAFB (November 20) (continuous-bagging treatment). The control fruit were not covered. Fruit from each treatment group (n = 10) were harvested periodically from 43 to 191 DAFB, and the aforementioned fruit-quality parameters were recorded.

**Effect of fruit bagging treatment period on good flesh and skin red coloration in ‘Kurenainoyume’ (Exp. 3)**

The experiment was conducted in 2012 using the same trees as those used in Exps. 1 and 2. Approximately 10 fruitlets from each tree were randomly covered with a 2-layer bag at 42 DAFB (June 24), and approximately two bags from each tree were periodically removed at 45 DBH (September 20), 35 DBH (September 30), 25 DBH (October 10), and 15 DBH (October 20), and all fruit were harvested at 175 DAFB (November 4). The control fruit were not covered. After harvesting, the aforementioned fruit-quality parameters were recorded.

**Statistical analysis**

Data were analyzed using the t-test, one-way analysis of variance (ANOVA), two-way ANOVA, or the Tukey–Kramer honestly significant difference (HSD) tests using JMP IN software (SAS Institute, Cary, NC, USA), and significant differences among the treatments were determined. Unless otherwise stated, differences were considered statistically significant at $P < 0.05$.

![Fig. 1. Effect of bagging treatment on red skin coloration, as evaluated by the $a^*$ value, on different types of red-fleshed apple cultivar, 'Goshogawara' (A) and 'Kurenainoyume' (B). Vertical bars represent standard error (SE; n = 10). ns, *, and ** indicate non-significant and significant differences at the 5 or 1% levels, by t-test or one-way ANOVA (n = 10). White and black arrows indicate the day of fruit bag covering or removal, respectively.](image-url)
was superior to that of continuous-bagging, suggesting light-dependent red coloration. The effectiveness of bag-removal was more obvious in ‘Kurenainoyume’ than ‘Goshogawara’ (Fig. 1). Continuous-bagging did not induce red skin coloration, although a slight increase in the a* value was observed in ‘Kurenainoyume’. It is worth noting that this slight increase could be ascribed to fruit flesh (inside flesh red coloration) rather than skin, because the non-colored white skin of ‘Kurenainoyume’ was very thin, which hampers the correct evaluation of skin a* values using a color difference meter.

Regarding core coloration, no notable differences in a* values were observed among the three treatments (non-bagging, continuous-bagging, and bag-removal) for the two types, although some significances were recorded between treatments on several sampling dates. Therefore, we summarized the changes in a* values in the core of each cultivar without distinguishing among the three bagging treatments. First, red coloration in the core indicated high a* values at the fruitlet stage. Then, a* values steadily decreased towards 125 DAFB, after which they remained relatively constant without notable changes until harvest stage in ‘Goshogawara’ (Fig. 2A). ‘Kurenainoyume’ did not show red core coloration under any of the three treatments (Fig. 2B), which is a typical feature of type 2 apples (Chagné et al., 2013; Umemura et al., 2013). The a* values of ‘Goshogawara’ flesh were high at the fruitlet stage and increased following a temporal decrease, remaining constant until harvest (Fig. 2C). In contrast, a* values in the flesh of ‘Kurenainoyume’ remained at low levels during the first 126 DAFB with all treatments. Then, the flesh coloration started to gradually increase, reaching similar a* values as ‘Goshogawara’ at the harvest stage (Fig. 2D). In both apple types, continuous-bagging resulted in lower a* values compared with the other two treatments at final harvest. These results demonstrated that there was no obvious effect of fruit bagging treatment on the red coloration of cores in ‘Goshogawara’ and no effect in ‘Kurenainoyume’, while the effect on flesh was somewhat light-dependent in both types (Fig. 2).

Next, we investigated the effects of light conditions on the flesh compounds. Concomitant with fruit maturation, the soluble solid and malic acid contents of ‘Goshogawara’ increased and decreased, respectively, although no statistical significances were found among the three treatments (Table 1). The firmness of ‘Goshogawara’ flesh significantly decreased with maturation, with an earlier decrease observed with non-bagging compared with the other two treatments. Changes in the soluble solid and malic acid contents of ‘Kurenainoyume’ followed the same patterns as observed in ‘Goshogawara’ with all three treatments (Table 1). At 177 DAFB (harvest stage), the soluble

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![Fig. 2.](image-url)

**Fig. 2.** Effect of bagging treatment on the core (A, B) and flesh (C, D) red coloration, as evaluated by the a* value, on different types of red-fleshed apple cultivar, ‘Goshogawara’ (A, C) and ‘Kurenainoyume’ (B, D). Vertical bars represent SE (n = 10). ns, *, and ** indicate non-significant and significant difference at the 5 or 1% levels, by t-test or one-way ANOVA (n = 10). White and black arrows indicate the day of fruit bag covering or removal, respectively.
solid and malic acid contents with the non-bagging treatment of ‘Kurenainoyume’ showed higher values than the other two treatments. In contrast, ‘Goshogawara’ did not show any difference in the soluble solid, malic acid contents, or flesh firmness between the treatments at harvest (146 DAFB). Flesh firmness of ‘Kurenainoyume’ significantly decreased with maturation; however, there were no significant differences among the treatments. Thus, the soluble solid and malic acid contents with non-bagging treatment of ‘Kurenainoyume’ were higher than with bag-removal and continuous-bagging; however, there were no significant differences in any parameters of ‘Goshogawara’ or in the flesh firmness of ‘Kurenainoyume’. In addition, there were no significant differences in the seasonal changes in fruit fresh weight for ‘Goshogawara’ and ‘Kurenainoyume’ on any sampling date, regardless of the treatment (data not shown).

Effect of fruit bagging treatment with different light permeabilities on the red coloration of skin, flesh, and core (Exp. 2)

Different types of paper bags with different degrees of light permeability have been developed for use in fruit bagging treatments (Matsumoto et al., 2018c), and each bag can differentially affect the external appearance, such as red coloration, depending on the fruit. To elucidate the effects of different light permeabilities on the skin, core, and flesh coloration, bag-removal and continuous-bagging treatments were employed for both type 1 and type 2 apples using three different types of paper bags, i.e., a 2-layer bag, a 1-layer white bag, or a 1-layer red bag.

As for skin coloration, changes in the a* value for the respective type generally showed similar trends as those observed with Exp. 1. At the fruitlet stage, ‘Goshogawara’ showed clear red coloration over the skin, and the red coloration then faded until 121 DAFB.
followed by a slight recovery at 139 DAFB (Fig. 3A). Bag-removal using red- and white-bags with some light permeability resulted in similar a* values as non-bagging; therefore, the bag-removal of red and white bags did not affect skin coloration, with the same values observed for non-bagging fruit. Similarly, bag-removal using a 2-layer bag, which completely prevented light penetration, also revealed similar a* values as those observed with non-bagging (Fig. 3A). Interestingly, even continuous-bagging induced an a* value at harvest, resulting in the same a* values at harvest regardless of the use of a 2-layer bag, a 1-layer white bag, or a 1-layer red bag. In Exp. 1, continuous-bagging using a 2-layer bag resulted in a significantly lower a* value without any increase observed at harvest, which differed from the result of Exp. 2. The reason for this difference was not clear, but may be due to year variations. We conclude that the skin color of ‘Goshogawara’ was only slightly induced by light.

Conversely, the skin in ‘Kurenainoyume’ fruitlets showed no red coloration, but red skin coloration was rapidly developed after 129 DAFB, except with continuous-bagging using a 2-layer bag (Fig. 3B). Continuous-bagging using a 1-layer white bag and a 1-layer red bag delayed the increase of red skin coloration; however, the extent of the red skin coloration was similar to other treatments except for the treatment with continuous-bagging using a 2-layer bag at the harvest stage. Thus, the skin of fruit subjected to continuous-bagging using a 2-layer bag did not show any coloration (Fig. 3B). Considering the positive results of 1-layer white and red bagging treatments, low light conditions were sufficient for good skin coloration of ‘Kurenainoyume’. These results suggested that light-sensitivity in the skin of ‘Goshogawara’ maybe weak, while that in ‘Kurenainoyume’ be strong (Fig. 3).

Regarding the core and flesh coloration, some differences in a* values were observed on several sampling dates among the treatments (non-bagging, continuous-bagging, and bag-removal) for both types of apple; however, the differences were small (Fig. 4). Red coloration of the ‘Goshogawara’ core indicated the highest a* values at the fruitlet stage, following which the a* values steadily decreased until harvest, 139 DAFB in all treatments (Fig. 4A). In contrast, ‘Kurenainoyume’ did not show any red coloration with any treatment (Fig. 4B). As for flesh, ‘Goshogawara’ showed the highest a* values at the fruitlet stage with all treatments, after which the a* values decreased with fruit maturation, and remained at constant levels after 100 DAFB, which was followed by a small increase at the harvest stage (Fig. 4C). The red coloration of ‘Kurenainoyume’ began from 129 DAFB and continued to the harvest stage; however, only continuous-bagging using a 2-layer bag resulted in lighter flesh coloration compared with other treatments at the final harvest stage (Fig. 4D). Therefore, we demonstrated that fruit bagging treatment had no obvious effect and no effect on red coloration of the respective ‘Goshogawara’ and ‘Kurenainoyume’ cores, while the effect on the flesh was somewhat light-dependent in both fruit types. However, except for continuous-bagging using a 2-layer bag, there were no obvious differences among the treatments.

Measurements of fruit flesh quality parameters, soluble solid content, malic acid content, and flesh firmness at harvest did not reveal any significant differences among the treatments in the two fruit types (data not shown).

Effect of fruit bagging treatment period on good flesh and skin red coloration in ‘Kurenainoyume’ (Exp. 3)

The results of Exps. 1 and 2 showed that fruit bagging treatment had no notable effect on the core red
coloration of 'Kurenainoyume' and 'Goshogawara' (Figs. 2A, B and 4A, B), while showing slight positive effects on red coloration of 'Kurenainoyume' and 'Goshogawara' flesh (Figs. 2C, D and 4C, D). 'Goshogawara' skin also showed red coloration by fruit bagging treatment, albeit only slightly (Figs. 1A and 3A). In contrast, we found a large impact of fruit bagging treatment on the skin coloration in 'Kurenainoyume' (Figs. 1B and 3B). Generally, fruit bagging treatment should not be applied to apple cultivation considering its cost-effectiveness. However, as mentioned earlier, fruit bagging treatment using a 2-layer bag is necessary to improve/prevent CSPD in 'Kurenainoyume' because it would not be possible for growers to produce apples of commercial value without the use of fruit bagging treatment (Matsumoto et al., 2018c). In contrast, since both 1-layer white and red bagging treatments developed some extent of CSPD, these two bags are not practically used for commercial cultivation (Matsumoto et al., 2018c). Therefore, the optimal bagging duration with a 2-layer bag has not yet been established even for skin coloration in 'Kurenainoyume'. In addition, it is difficult for growers to directly determine the degree of flesh coloration from the outside of the fruit. Therefore, it is necessary to optimize the conditions of fruit bagging treatments, considering not only CSPD, but also the red coloration of skin and flesh, in 'Kurenainoyume'. For practical purposes, we attempted to determine the timing of bag-removal to obtain the highest degree of red coloration in the skin and flesh in 'Kurenainoyume'.

Additional red colorations of the flesh were recorded following bag-removal at 25, 35, and 45 DBH compared with the non-bagging control (Fig. 5). Interestingly, red coloration in the flesh was observed even with continuous-bagging, although the intensity was the same as control and lower compared with other treatments except bag-removal at 15 DBH. The same tendency was also observed in both Exps. 1 (Fig. 2) and 2 (Fig. 4), in which the continuous-bagging treatment indicated significantly lower red coloration than the control and bag-removal treatments at final harvest. Thus, bag-removal can improve red coloration of 'Kurenainoyume' flesh, especially with bag-removal at 25, 35, and 45 DBH (Fig. 5), although it seemed to show year to year variations. Contrary to flesh coloration, skin color revealed large variations in 'Kurenainoyume': i) continuous-bagging did not cause the development of any skin coloration, ii) non-bagging led to maximum red coloration, and iii) skin coloration became deeper and deeper with a longer period from bag-removal to harvest (Fig. 6). Here, it is noteworthy...
to observe CSPD in the skin of non-bagging fruit (Fig. 6), which suggests the necessity of fruit bagging treatment for ‘Kurenainoyume’, although the best result among the treatments was observed for skin coloration. With the exception of non-bagging, bag-removal at 25, 35, and 45 DBH, resulted in good skin coloration; those treatments also resulted in the most suitable flesh coloration (Fig. 5). Moreover, there were no significant differences in fresh weight, soluble solid, and malic acid contents compared with the non-bagging control (Table 2). Flesh firmness was higher with bag-removal at 25 DBH treatment compared with the other treatments, although this fact did not lead to reduced fruit quality (Table 2). Taking into consideration the results shown in Figures 5 and 6, and Table 2, we recommend bag-removal from 25 to 45 DBH for ‘Kurenainoyume’ cultivation.

**Discussion**

In Japan, fruit bagging treatment is a common cultivation technique in apple production, and is used to improve fruit qualities, especially skin appearance, i.e. red coloration (Arakawa and Komori, 2006). In non-red cultivars, ‘Mutsu’ and ‘Kinsei’, the fruits showing clear red coloration, or part blushing as a result of fruit bagging treatment, can be sold at a high price. Therefore, fruit bagging treatment has been used by growers, despite the costly and labor-intensive procedures. However, considering cost-effectiveness, fruit bagging treatment should not be a first choice for practical application. Regarding ‘Kurenainoyume’, growers cannot produce fruits with high commercial value without application of fruit bagging treatment because this cultivar type exhibited CSPD without fruit bagging treatment using a 2-layer bag (Fig. 6). Moreover, some ‘Kurenainoyume’ apple growers have applied fruit bagging treatment to enhance storability; in fact, bagged

### Table 2. Effect of bag-removal timing for the fresh weight, soluble solid content, titratable acidity, and flesh firmness of ‘Kurenainoyume’ apples.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g)</th>
<th>Soluble solid content (°Brix)</th>
<th>Malic acid (mg/100 mL)</th>
<th>Flesh firmness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-bagging</td>
<td>283.7 a</td>
<td>12.1 a</td>
<td>0.86 a</td>
<td>71.0 b</td>
</tr>
<tr>
<td>Bag-removal at 45 DBH</td>
<td>262.3 a</td>
<td>11.4 a</td>
<td>0.88 a</td>
<td>75.0 b</td>
</tr>
<tr>
<td>Bag-removal at 35 DBH</td>
<td>293.5 a</td>
<td>11.7 a</td>
<td>0.80 a</td>
<td>71.9 b</td>
</tr>
<tr>
<td>Bag-removal at 25 DBH</td>
<td>249.2 a</td>
<td>12.4 a</td>
<td>0.90 a</td>
<td>87.6 a</td>
</tr>
<tr>
<td>Bag-removal at 15 DBH</td>
<td>238.3 a</td>
<td>12.2 a</td>
<td>0.80 a</td>
<td>78.7 ab</td>
</tr>
<tr>
<td>Continuous-bagging</td>
<td>258.7 a</td>
<td>12.3 a</td>
<td>0.84 a</td>
<td>77.0 b</td>
</tr>
</tbody>
</table>

Different letters within the same column indicate a significant difference by Tukey-Kramer’s HSD test at the 5% level (n = 5).

DBH indicates the day before harvest.
fruit showed longer storage periods compared with non-bagging (Matsumoto et al., 2018a). ‘Kurenainoyume’ also has potential for additional processing due to the characteristic red flesh coloration. Indeed, some companies in Aomori Prefecture have begun to develop juice using ‘Kurenainoyume’, as well as jam, dry fruit, pies, and tarts (Matsumoto, 2017). Accordingly, the fruit can be sold at a high price that makes it possible to compensate for the additional cost and effort required for fruit bagging treatment. As one instance, when the net income from ‘Fuji’ apples was compared with non-bagging and bag-removal treatments, the fruit obtained with bag-removal could be sold at a higher price than those with non-bagging treatment, resulting in about a 400000 yen/10a higher return (Aomori Prefectural Government, 2010). Under these conditions, we aimed to develop a fruit bagging treatment suitable for application to the commercial production of ‘Kurenainoyume’ fruit. In addition to the improvements in skin and flesh coloration, the results showing fresh weight, soluble solid contents, malic acid contents, and flesh firmness with fruit bagging treatment were comparable to those of non-bagging treatment (Table 1), and are also of great value to develop practical cultivation techniques. Therefore, we recommend bag-removal from 25 to 45 DBH for commercial ‘Kurenainoyume’ cultivation, and we believe that the techniques described in this study will enable growers of ‘Kurenainoyume’ to generate a significant increase in net income.

Anthocyanin biosynthesis in apples is mainly regulated by two factors, genetic and environmental cues (Ubi et al., 2006). Our knowledge of the genetic aspects (=molecular mechanism) of apple red coloration, not only in the skin but also in the flesh, has increased since the discovery of MYB (e.g. Peng and Moriguchi, 2013). Studies on both basic and practical aspects of environmental factors for red coloration have also been carried out, including nutrients, wounding, pathogen infection, water stress, temperature, and UV light (Chalker-Scott, 1999; Dixon and Paiva, 1995). Among various environmental stimuli, UV-B is one of the main factors affecting anthocyanin accumulation (Ban et al., 2007; Ubi et al., 2006). Indeed, skin coloration of type 2 ‘Kurenainoyume’ occurred in a light-dependent manner, with much greater skin coloration in the non-bagging treatment compared with continuous-bagging (Figs. 1B, 3B, and 6). Bag-removal resulted in the highest a* values among the three treatments (Figs. 1B, 3B, and 6). Thus, ‘Kurenainoyume’ showed high sensitivity to light, where even faint light was sufficient for red skin coloration. By contrast, red coloration of type 1 ‘Goshogawara’ skin tended to be light-independent because light could improve red coloration in both non-bagging and bag-removal treatments in some extent, compared with continuous-bagging (Fig. 1A). However, no light effect difference was clearly observed between non-bagging and bag-removal treatments (Fig. 3A). We expected an improvement in the skin coloration of type 1 ‘Goshogawara’ with bagging treatment because this cultivar possesses a strong R6 promoter in the MYB gene (Espley et al., 2009); therefore, we cannot give a clear explanation to the unexpected lack of effect at present. Regarding the core coloration of ‘Goshogawara’, light treatments, i.e. non-bagging and bag-removal, did not show obvious effects (Figs. 2A and 4A), while the core coloration of ‘Kurenainoyume’ was not recorded even with bagging treatments (Figs. 2B, 4B, and 5). Conversely, flesh coloration of ‘Kurenainoyume’ was improved with bag-removal compared with continuous-bagging at the harvest stage, albeit only slightly (Figs. 2D, 4D, and 5). Similarly, flesh coloration of ‘Goshogawara’ was improved with bag-removal compared with continuous-bagging at the harvest stage (Figs. 2C and 4C), but the effect of light on the flesh may be smaller than the skin in both ‘Goshogawara’ and ‘Kurenainoyume’. Honda et al. (2017) also reported that sunlight irradiation partially promoted anthocyanin accumulation in the flesh of non-bagging ‘Geneva’ (type 1) and ‘Pink Pearl’ (type 2) apples compared with bagging treatment (corresponding to continuous-bagging in our study); although there were differences between the non-bagging control and continuous-bagging in both cultivars with being some year to year variations (Honda et al., 2017). Similarly, red coloration of the flesh of both ‘Goshogawara’ and ‘Kurenainoyume’ tended to be light-dependent, but the extent showed year to year variations that were less sensitive than the skin.

It has been reported that red coloration in flesh and core of type 1 apples is ascribed to the strong R6 promoter activity of MdMYB10R6; thereby, it is hypothesized that red coloration can occur in the tissues of type 1 apples even under dark conditions. The flesh coloration in type 2 ‘Kurenainoyume’ is ascribed to MdMYB110a. Then, we assumed that light is necessary for red flesh coloration as in the case of MdMYB110a. Indeed, light showed a positive effect on the flesh coloration to some extent in ‘Kurenainoyume’ (Figs. 2, 4, and 5). The slight improvement in flesh red coloration by bag-removal may be due to the epigenetic regulation of MdMYB genes such as methylation and chromatin modification (Bai et al., 2016). Bai et al. (2016) reported higher levels of histone H3 acetylation and trimethylation of its tail at lysine 4, and a lower level of trimethylation of this tail at lysine 27 was observed in the 5′upstream region of MdMYB1-2/-3 after bag-removal in the yellow apple ‘Mutsu’, along with increases in the DNA demethylation level of MdMYB, all of which could facilitate the binding of trans factor(s) to MdMYB and result in the activation of these MYBs after paper bag-removal, although the red flesh coloration was also recorded in continuous-bagging treatment (Figs. 2, 4, and 5). It is also possible that signal transduction pathways of light in MdMYB110a are
completely different from those of *MdMYBA/110*. However, we could not clearly address these contradictory results at present, and further in-depth studies using many cultivars of both types are needed.

It is generally known that the contents and compositions of secondary metabolites including anthocyanins show annual variations (Lata, 2007; Lata and Tomala, 2007). Indeed, annual and/or locational differences in the levels of red coloration of ‘Kurenainoyume’ flesh have been reported (Matsumoto, 2017). In general, skin color as well as flesh color tends to deteriorate in apple trees with active vegetative growth, which indicates that too much soil nitrogen has a negative impact on anthocyanin accumulation. In addition, cooler climate conditions are advantageous for flesh coloration in ‘Kurenainoyume’ (Matsumoto et al., 2018b). Honda et al. (2014, 2017) also showed the effect of cooler temperature on the flesh red coloration in ‘Pink Pearl’. The practical technique of fruit bagging treatment described in this study has been widely utilized by growers in Aomori Prefecture for several years. The results showed stable production of high quality ‘Kurenainoyume’ apples with good skin and flesh coloration, regardless of year or location. Accordingly, we believe that the techniques described in this study will enable growers of ‘Kurenainoyume’ to generate a significant increase in net income.

We investigated the effects of light conditions on the red coloration of type 1 and type 2 apples in this study, and found that red coloration in apples was not regulated only by light conditions. We therefore intend to investigate factors other than light conditions responsible for flesh red coloration in future studies.

**Literature Cited**


Matsumoto, K., T. Fujita and S. Sato. 2018a. Effects of 1-MCP and pre-harvest fruit bagging treatments on cold storability


