How to manipulate hydrangea flower colour (*Hydrangea macrophylla* Thunb.)?

E.G. Ergür[1,a], S. Kazaz[1], T. Kılıç[2], E. Doğan[3] and B. Aslansoy[4]

[1]Department of Horticulture, Faculty of Agriculture, Ankara University, 06130 Ankara, Turkey; [2]Department of Horticulture, Faculty of Agriculture, Bozok University, 66200 Yozgat, Turkey; [3]Department of Horticulture, Faculty of Agriculture, Bingöl University, 12000 Bingöl, Turkey; [4]Plant Biodiversity, Geophyte Research and Training Centre Directorate, 37820 İstanbul, Turkey.

Abstract

In recent years, hydrangea (*Hydrangea macrophylla*), which has common usage as cut flowers, potted plants and garden plants, has been gradually increasing in both popularity and production in the world. The colour of hydrangea's sepals varies from blue to purple and red depending on soil pH, sucrose content, metal content as well as temperature. It is possible to see different colours in the flowers which are located on the different stems of the same plant depending on the pH value of the growth medium and the additional aluminium (Al) level. When the pH value of the growth medium is between 4.5 and 5.0, the uptake ability of Al increases and the colour of the sepals becomes blue. In the event that pH value is 6.5, the uptake of Al becomes restricted and the sepals turn into pink and red colour. In neutral conditions, the sepals are purple. Anthocyanin has a particular importance on the colour change of hydrangeas. In the structure of these compounds, as the number of -OH group (hydroxyl) increases, the blue colour increases; but as the number of OCH_3 groups (methoxylated hydroxyl) increases, the redness increases. Anthocyanin, delphinidin 3-glucoside and co-pigments have an effect on blueness in hydrangeas. Both pink and blue sepals contain the same anthocyanin pigment (delphinidin 3-monoglucoside). Al in the sepals binds the pigments and 3-caffeoyl and 3-p-coumaroylquinic acids serving as co-pigments, and this complex formation of Al/pigment/co-pigment is the reason for the colour transition of sepals from pink to blue. In this report, information about manipulation of flower colour is provided.

Keywords: anthocyanin, pigments, metal content, pH, aluminium

INTRODUCTION

Hydrangea is a species of *Hydrangeaceae* family (Chhune, 2015). The *Hydrangea* genus includes 90 species (Bailey, 1992), but there are 23 species dispersed in both temperate and tropical regions of East Asia, North East America and South America. The most popular one in these species is *H. macrophylla* (Kardos et al., 2009), originated in South China and Japan. Hydrangeas are northern hemisphere plants that spread throughout eastern Asia, especially China and Japan (Kardos et al., 2009; Nesi et al., 2013). *H. macrophylla*, is a deciduous shrub which grows vertically to 1-1.5 m. The petiolar and thick leaves reach 7-15 cm and line up oppositely. The flowers grow on the branches of the previous year and bloom from the beginning of spring until the end of autumn (http://www.quincunx.es/2013/08/08/hortensias; Nesi et al., 2013). Flower colour is usually blue, pink or purple, but rarely white. There are two types of inflorescences, mophead (var. *macrophylla*) and lacecap (*H. macrophylla* var. *normallis*), the former one comprises large, globe-shaped and completely sterile flowers, while the latter inflorescence type, has fertile flowers in the centre, surrounded by outer rings of large and sterile flowers. In sterile flowers, there are 4-5 large petaloid sepals, 4-5 small petals, 8-10 stamens and semi-ovarian which has 3-4 small-short stamens (Illan, 2011). Seasonal or evergreen hydrangea can form bush, climbing and even small trees, used as ornamental plants as potted plants, cut flowers or in garden decoration

[a]E-mail: gozde_gop@hotmail.com

(Nesi et al., 2013). Hydrangea has commercial use as dry and fresh cut flowers (Arango, 2003). Both the popularity and production of hydrangea has increased in the world in recent years.

It is known that the sepals of *H. macrophylla* flowers change colour depending on the growing conditions. All coloured hydrangea sepals have only a single anthocyanin pigment (delphinidin 3-glucoside). Depending on the level of pH, the anthocyanin changes the colour of the hydrangea (Ito et al., 2009). Hydrangea has a broad colour range from blue to purple pink and red. Only the white species do not change their colour and all the flowers age and finally turn green. Sepal colour of hydrangea transforms depending on many physical and chemical factors, such as the stability of the anthocyanins, the chemical structure, the presence of co-pigment, the pH value of the growing medium, temperature, enzyme presence and metal ion.

FACTORS AFFECTING COLOUR VARIATION IN HYDRANGEA

Genotype

Colour variation in hydrangea differs depending on its cultivars. Although some cultivars are better suited for blue colour, others are better in pink or red colour. Hence, when a certain colour is desired in cultivation, it is better to choose a cultivar which provides this type of colour. For example, for dark red 'Böttstein' and 'Schenkenburg'; for dark pink 'Kasteln' and 'Merritt's Supreme', and for light pink 'Rose Supreme' and 'Enziandom' cultivars should be preferred (Bailey, 1914) (Table 1).

Table 1. Sepal colour description of the most common hydrangea cultivars.

Cultivar	Grown as a pink	Grown as a blue
Böttstein	Dark red	Not recommended
Brestenburg	Not recommended	Medium blue
Enziandom	Light pink	Deep blue
Kasteln	Dark pink	Medium blue
Mathilda Gütges	Not recommended	Light blue
Merritt's Supreme	Dark pink	Medium blue
Red Star	Light red	Medium blue
Rose Supreme	Light pink	Light blue
Schenkenburg	Dark red	Not recommended
Sister Therese	White	White

Anthocyanins

Anthocyanin is natural pigment which is found in plant organs such as fruits, flowers, leaves and roots; with a wide range of colour to the plant as pink, red, purple and blue (Chandra et al., 2001; Blando et al., 2004). In plant tissues, anthocyanins are synthesized at different concentrations depending on the genetics of the plant, the environmental factors during its growth, the stress conditions the plant is exposed to, the amount of water, the presence and amount of minerals and organic compounds in the soil. In addition, the growing year, maturity level, the postharvest storage period and also temperature were found to be effective on the amount of anthocyanin (Gonçalves et al., 2004). The stability of anthocyanins is generally affected by many physical and chemical factors such as chemical structure, presence of co-pigment, pH value, temperature, presence of enzyme (polyphenol oxidase and peroxidase) and metal ion (Chandra et al., 1993; Cemeroğlu et al., 1994).

Chemical structure

The presence of linked sucrose shifts methoxylation of ring-B (OCH3), level of hydroxylation (OH) and the presence of acyl groups affect pigment stability and colour intensity (Giusti et al., 1999). While methoxylation increases stability, hydroxylation decreases.

Sucrose amount

Anthocyanins are found in the flavonoid group of phenolic compounds and are chemically glycosidic. The aglycone portion of the glucoside is generally called anthocyanidins and different anthocyanins are formed by the glycosidic attachment of different saccharides to the anthocyanidins (Mazza and Miniati, 1993). Anthocyanins are found in glycoside form which anthocyanin forms with sucrose in plants (Clifford, 2000). The colour difference between the various anthocyanins changes depending on the number of -OH group (hydroxyl group) in the molecule, the degree of methylation of the hydroxyl groups, the number of molecules linked to the sucrose and the sucrose linking position, the cinnamic acids (caffeic, p-kumaric, ferulic and sinapic) linked to sucrose, aliphatic (acetic, malic, malonic, oxalic and succinic) and the structure (Mazza and Miniati, 1993) and the number of aromatic acids and more than 500 different anthocyanins occur (Castañeda-Ovando et al., 2009).

Presence of co-pigment

Co-pigment forms a complex with anthocyanins and produces different coloured compounds. These substances prevent anthocyanins from forming colourless carbonyl forms by stabilizing their coloured forms, despite appropriate pH value (Cemeroğlu et al., 2004).

Co-pigments, which are usually colourless compounds, protect the anthocyanins against hydration and stabilize the colour. The main/primary/leading compounds behaving like co-pigment are flavonoids, polyphenols, alkaloids, amino acids and organic acids (Chandra et al., 1993). The concentration and structure of the anthocyanin incorporated into the co-pigmentation, the concentration and structure of the co-pigment, the pH and the temperature of the medium and of the solvent effect sensitivity of formation of co-pigment (Mazza and Miniati, 1993). In anthocyanins, the co-pigmentation takes place mainly in three different ways.

The first one is self-association, that is interaction which takes place through an intramolecular between the anthocyanidin core and the aromatic acyl groups; second one is intramolecular co-pigmentation which take place through interactions between anthocyanidin core and non-chromophore sections (aromatic acid) of the molecule; and the third one is the intermolecular co-pigmentation which take place between two or more molecules (Kırca, 2004; Castañeda-Ovando et al., 2009).

All coloured sepals contain the same anthocyanin pigment (delphinidin 3-monoglucoside) and the same co-pigment compounds which are chlorogenic acid (3-O-caffeoylquinic acids), neochlorogenic acid (5-O-caffeoylquinic acids) and 5-Op-coumaroylquinic acid (Ito et al., 2009). Some qualitative observations have revealed that anthocyanin delphinidin 3-glucoside and co-pigments in blue sepals defined as 3-caffeoyl- and 3-p-coumaroylquinic acids may cause blueness for hydrangeas. Aluminium in sepals binds pigment and co-pigments which are 3-caffeoyl and 3-p-coumaroylquinic acids and this aluminium/pigment/co-pigment complex change the colours of sepals from pink to blue (Takeda et al., 1985). Co-pigments are necessary to stabilize the Al-delphinidine complex (Schreiber et al., 2010). As a co-pigment, 5-caffeoylquinic acid (chlorogenic acid) is less effective in changing blue colour, because it forms purple rather than blue colour. It is also reported that both compounds are required for complete blue colour formation, although aluminium has a greater effect on the formation of blue colour than 5-caffeoylquinic acid (Takeda et al., 1985).

pH value

An additional factor affecting the colour stability of anthocyanins in aqueous medium is the pH value (Mazza and Miniati, 1993) and the anthocyanins are converted into different chemical forms depending on the pH value of the solution. At pH 1, flaviolium cation in red colour is dominant and contributes to purple-red colour formation. On the other hand, while at pH 2-4 quiniodal base becomes predominant and provide to occur blue colour; at pH 5-6 colourless structures called carbinol pseudobase and calcon are formed (Castañeda-Ovando et al., 2009). In this range, the anthocyanins are highly unstable and rapid colour changes occur due to the hydration of the 2nd position of the anthocyanidin structure (Ersus and

Yurdagel, 2006). If the pH value is higher than 7, like anthocyanins, they are disintegrated into their constituent compounds (Castañeda-Ovando et al., 2009). It is necessary to know that pH value of the growing medium and which pH range of the growing medium influences aluminium (Al) and iron uptake to change colour in Hydrangea. Intake of Al is decisive in colour formation in sepals except for white species (Bailey, 1992). Intake of aluminium is directly related to the pH of the growing medium. When the pH of the growing medium is 4.5-5, the intake of Al increases and sepals become blue colour, but when the pH of the growing medium is 6-6.5, the uptake of Al is restricted and sepals become pink (Bailey, 1992; Boztok, 200; Illan, 2011).

The hue of anthocyanin changes depending on pH value. Sepals turn into blue in high acidic conditions, turn purple in neutral conditions and red colour in alkaline conditions. However, the vacuolar pH of plant cells is generally low acidic, and under these conditions almost all anthocyanins are purple (Yoshida et al., 2003). In the acidic soil, the root system of the hydrangea releases citric acid in the soil, and the citric acid in the soil reacts with aluminium to form a complex. In acidic soils, Al^{3+} dissolves, then it is absorbed by the roots and finally it is transmitted to the sepals. In sepals, blue colour formation is obtained by the complexation of anthocyanin with the Al^{3+} ions (Ito et al., 2009). Because $Al(OH)_3$ precipitates the basic soil, aluminium cannot be taken up by the plant and therefore the anthocyanin colour remains red in these sepals (Schreiber et al., 2010).

Temperature

The process and storage temperature is one of the most important factors affecting the decomposition of anthocyanins. There is a linear correlation between increase in temperature and disintegration rate of the pigments (Seeram et al., 2001). The effects of temperature on the decomposition of anthocyanins in cherry juice and pomegranate juice concentrates were studied and it has been reported that as the storage temperature increases, the pace of degradation of anthocyanins increases (Asafi and Cemeroğlu, 2000).

The presence of oxygen in the medium transforms the brilliant colour of anthocyanins into brown, and oxygen and heat become effective in the degradation of anthocyanins.

Presence of enzyme

Enzyme presence in medium is another cause that affects the stability of anthocyanins. Glycosidase (anthocyanase) hydrolyzes the glycosidic linkages existing in anthocyanins, resulting in unstable anthocyanidins, and bleaching of the colour (Seeram et al., 2001). Other than glycosidases, polyphenol oxidase and peroxidase catalysed reactions affect the anthocyanins (Chandra et al., 1993). Anthocyanins react with the o-quinones, which are the result of the oxidation of other phenolics. The unstable quinones of the anthocyanins are formed by oxidation of the anthocyanins that are o-diphenolic structure. These reactions cause significant loss of colour (Ersus and Yurdagel, 2006).

Metal ions

Many anthocyanins form complexes with several divalent metal ions such as iron, aluminium, copper, and thus the unstable red colour is transformed into a stable blue (Cemeroğlu et al., 2004). Anthocyanin-metal complex is a very important interaction in terms of colour stabilization. The blue colour remains stable due to the protection from oxidation both Al (III) -anthocyanide interactions and the quiniodial base (Moncada et al., 2003). The anthocyanin-molybdenum complex formed by the addition of Mo (IV and VI) to the tissue increases the stabilization of the blue colour (Castañeda-Ovando et al., 2009). In the NaCl solution, while the colour stabilization increases thanks to the spontaneous association of anthocyanins, in $MgCl_2$ solution, it lessens due to the hydration of magnesium ions and decreased free water (Mazza and Miniati, 1993).

All coloured hydrangea sepals, except for white ones, have a red pigment which turns blue by the effect of aluminium. For this reason the relative availability of Al is the main determinant of the colour of the flowers. Therefore, pink flowers eventually become blue unless precautions are taken to prevent Al absorption. Similarly, if there is not enough Al to

react with enough of the anthocyanin pigment, a bluish-purple colour forms in most forms (Arango, 2003; Illan, 2011). Flower colour can be controlled by Al formation (Bailey, 1914). For blue species, physiological acid fertilizers should be preferred in order to stabilize the pH of growing medium and at large amount of free-aluminium (Al) should also be present. Because, Al^{3+} blocks uptake of phosphorus. Potassium is also required at high concentrations (www.infoagro.com/flores/plantas ornamentales/hortensia.htm; Bailey, 1914).

The ability of the hydrangea to produce blue or pink colour depends on the availability and uptake of aluminium, except for white varieties (Bailey, 1914). While Al promotes the transformation of the pigment of the anthocyanin to blue colour, it protects the intracellular pH from changes maintaining and allows this colour to remain constant. The blue colour flowers are obtained from the pink coloured cultivars containing the delphinine group anthocyanin which is capable of turn into blue in some special conditions. Because they contain more colour material in the vacuole water and to provide full colour transformation, darker coloured varieties need more to contain Al. The presence of aluminium is directly dependent on the pH of the soil solution: aluminium uptake is high between 5 and 5.5 and the sepals may be blue; but, it remain as pink colour at 6.0-6.5 (Takeda et al., 1985; Bailey, 1992). Al, which forms complexes with delphinidin-3-glucoside, is the main factor to turn in blue colour in hydrangea sepals. For the formation of the complex, aluminium removes H^+ ions from delphinidine-3-glucoside and converts from the normally red-coloured flavylium cation of delphinidine to a blue quinoidal-based anion complexed with aluminium. The formation of the complex increases the blueing (Schreiber et al., 2010).

If it is desired to obtain pink coloured flowers, it should be avoided to give aluminium to plants and mineral soil should not be used in the growing median. Phosphorus helps to form pink inflorescence, preventing the uptake of aluminium. High nitrogen, high phosphorus and low potassium promotes the formation of light pink coloured sepals and 25-10-10 (N-P_2O_5-K_2O) fertilization is recommended (Bailey, 1992). Although there is enough Al in the environment, if combined with high potassium, the low levels of both elements promote the formation of a light blue coloured sepal, while the higher levels promote pink sepal formation. For this reason, if pink colour is desired in the potted hydrangea cultivation, Al should not be used in the growing medium. Addition of triple superphosphate into the medium and addition of mono-ammonium-phosphate (11-53-00) to the feed schedule contributes to an increase in the phosphorus level. At low pH, it is become easy to uptake aluminium. For pink-coloured flowers, the pH of the solution should be adjusted between 6.0 and 6.2. If the pH of irrigation water is higher than 6.5, acidification should be done. Phosphoric acid should be used as it increases the phosphorus level as an acidifier. In the blue hydrangea cultivation aluminium is applied over 100 ppm with irrigation water. The formation of blue coloured seals is achieved by using high amounts of potassium, medium nitrogen and low amounts of phosphorus (Bailey, 1914, 1992) and pH of irrigation water should be 5-5.2 (Illan, 2011).

Aluminium sulphate should be given to the medium to get blue-coloured hydrangea. If the pH of the irrigation water is higher than 5.8, 35% sulphuric acid which is the best acidifier should be added to reduce the pH. Higher doses and phosphorus-free or low-dose phosphorous fertilizers should be used for blueing (Bailey, 1914). The pH should be maintained between 6 and 6.2; because this low acidity facilitates the use of aluminium while at high pH forms ferric chlorosis. In order to reduce the pH level, it is best to use phosphoric acid. In order to maintain the pH level of the irrigation water at 5-5.2, sulphuric acid and moderate amounts of nitrogen, high potassium and low levels of phosphorus should be used. Acidic fertilizers like ammonium sulphate, ammonium nitrate, potassium sulphate should be given for blue flowers. Aluminium sulphate can also be added where it is inadequate (Blom and Piott, 1992). Iron intake is affected by the pH level of the growing media as well. Hydrangeas are sensitive to iron deficiency chlorosis and chlorosis increase especially at pH above 6.0 (Bailey, 1914, 1992; Illan, 2011).

CONCLUSIONS

The use of hydrangea in the ornamental plant industry has become increasingly widespread in recent years. *H. macrophylla* is the most commercial and popular species

among the *Hydrangea* species. Sepal colours of this species changes from blue to purple and red colour depending mainly on pH value of the growing medium and Al contents as well as sugar, metals, co-pigments and temperature, and thus it is possible to see different colours on different branches of the same plant together. When the pH value of the cultivation medium is between 4.5 and 5.0 sepal colours turns into blue, it becomes pink and red colour at 6.5, it gets purple in neutral conditions.

Sepal colour is very famous for ready change of hues. However, although it is well-known that acidity of the soil and content of Al^{3+} influence sepal colour, the underlying mechanism remains unclear (Pecchioli et al., 2013).

In the study, which aims to understand mechanism providing blue and red colour in hydrangea, delphinidin-3-O-β-D glucopyranoside and co-pigment compounds, 5-O-caffeoyl quinic asit, 5-O-p-coumaroly quinic acid and 3-O-caffeoyl quinic acid are isolated from hydrangea sepals. It has been found that vacuolar pH in blue hydrangea sepals is higher than red sepals. Although the reason why there are pH differences between red and blue cells is not understand properly, it is explained that this difference could be related with inequality in amount of Al. Because there are ten times Al in blue sepal cells than red sepal cells. Furthermore, molar ratio for co-pigments and Al^{3+} may have an effect on sepal colour (Yoshida et al., 2003). It has been found that blue sepals contain much more 3-caffeoyl and 3-p-coumaroylquinic acid and contain less 5-caffeoylquinic, while amount of delphinidin 3-glucoside is equal in both colours. The effect of 3- and 5-caffeoylquinic acid on making colour (co-pigmentation) is negligible quantity. Even though Al^{3+} has much more effect than 3-caffeoylquinic acid when molar ratios are used, the study shows that it is necessary to use both compounds to make a pure blue colour (Takeda et al., 1985). Hence, it is considered that colour variation of hydrangea sepals may depend on controlling all these factors.

Literature cited

Arango, M. (2003). *Hydrangea hortensia*. Ed. Hortitecnia Ltda. P. *7–8*, 21–24.

Asafi, N., and Cemeroğlu, B. (2000). Gida *25* (*6*), 407–411p.

Bailey, D.A. (1914). Commercial Hydrangea Forcing (Department of Horticultural Science), p.1–4.

Bailey, D.A. (1992). Hydrangeas. Introductıon to Floriculture, 2nd edn (San Diego, California: Department of Horticultural Science, College of Agriculture and Life Sciences, Academic Press), p.365–383.

Blando, F., Gerardi, C., and Nicoletti, I. (2004). Sour cherry (*Prunus cerasus* L) anthocyanins as ingredients for functional foods. J. Biomed. Biotechnol. *2004* (*5*), 253–258 https://doi.org/10.1155/S1110724304404136. PubMed

Blom, T.J., and Piott, B.D. (1992). Florists' Hydrangea Bluening with Aluminum Sulfate Applications during Forcing (Vineland Station, Ont., Canada: Ontario Ministry of Agriculture and Food, Horticultural Research Institute of Ontario).

Castañeda-Ovando, A., Pacheco-Hernàndez, M.L., Pàez-Hernàndez, M.E., Rodriguez, J.A., and Galàn-Vidal, C.A. (2009). Chemical studies of anthocyanins: a review. Food Chem. *113* (*4*), 859–871 https://doi.org/10.1016/j.foodchem.2008.09.001.

Cemeroğlu, B., Velioğlu, S., and Işık, S. (1994). Degradation kinetics of anthocyanins in sour cherry juice and concentrate. J. Food Sci. *59* (*6*), 1216–1218 https://doi.org/10.1111/j.1365-2621.1994.tb14680.x.

Cemeroğlu, B., Yemenicioğlu, A., and Özkan, M. (2004). Meyve ve sebzelerin bileşimi. Meyve ve Sebze İşleme Teknolojisi, I. Cilt, and B. Cemeroğlu, eds. (Ankara: Bizim Büro Basımevi), p.1–188.

Chandra, A., Nair, M.G., and Iezzoni, A.F. (1993). Isolation and stabilization of anthocyanins from tart cherries (*Prunus cerasus* L.). J. Agric. Food Chem. *41* (*7*), 1062–1065 https://doi.org/10.1021/jf00031a009.

Chandra, A., Rana, J., and Li, Y. (2001). Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. J. Agric. Food Chem. *49* (*8*), 3515–3521 https://doi.org/10.1021/jf010389p. PubMed

Chhune, T. (2015). Studies on postharvest handling of cut hydrangea (*Hydrangea macrophylla* Thunb.) as influenced by pre-harvest fertilization. Thesis (India: Dr. Yashwant Singh Parmar University of Horticulture and Forestry).

Clifford, M.N. (2000). Anthocyanins – nature, occurrence and dietary Burden. J. Sci. Food Agric. *80* (*7*), 1063–1072

https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1063:AID-JSFA605>3.0.CO;2-Q.

Ersus, S., and Yurdagel, Ü. (2006). Meyve ve Sebzelerde Bulunan Antosiyaninlerin Kimyasal Yapısı ve Stabilitesini Etkileyen Faktörler (Chemical composition and factors affecting the stability of anthocyanins in fruit and vegetables). Dünya Gıda *10*, 78–87.

Giusti, M.M., Rodríguez-Saona, L.E., and Wrolstad, R.E. (1999). Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. J. Agric. Food Chem. *47* (*11*), 4631–4637 https://doi.org/10.1021/jf981271k. PubMed

Gonçalves, B., Landbo, A.K., Knudsen, D., Silva, A.P., Moutinho-Pereira, J., Rosa, E., and Meyer, A.S. (2004). Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). J. Agric. Food Chem. *52* (*3*), 523–530 https://doi.org/10.1021/jf030595s. PubMed

Illan, R.V. (2011). Cultivo de hortensia en maceta bajo agua residual depurada salina: eficacia del lavado con agua buena. Master Tezi (Cartagena: Departamento de Produccıon Vegetal, Escuela Tecnica Superior de Ingenieria Agronomica, Universidad Politecnica de Cartagena), pp.96.

Ito, D., Shinkai, Y., Kato, Y., Kondo, T., and Yoshida, K. (2009). Chemical studies on different color development in blue- and red-colored sepal cells of *Hydrangea macrophylla.* Biosci. Biotechnol. Biochem. *73* (*5*), 1054–1059 https://doi.org/10.1271/bbb.80831. PubMed

Kardos, J.H., Robacker, C.D., Dirr, M.A., and Rinehart, T.A. (2009). Production and verification of *Hydrangea macrophylla* × *H. angustipetala* hybrids. HortSci. *44* (*6*), 1534–1537.

Kırca, A. (2004). Siyah havuç antosiyaninlerinin bazı meyve ürünlerinde ısıl stabilitesi. Doktora tezi (basılmamış) (Ankara: Ankara Üniversitesi Fen Bilimleri Enstitüsü).

Mazza, G., and Miniati, E. (1993). Anthocyanins in Fruits, Vegetables and Grains (London: CRC Press), pp.362.

Moncada, M.C., Moura, S.M., Melo, J., Roque, A., Lodeiro, C., and Pina, F. (2003). Complexation of aluminum(III) by anthocyanins and synthetic flavylium salts. Inorg. Chim. Acta *356*, 51–61 https://doi.org/10.1016/S0020-1693(03)00394-3.

Nesi, B., Lazzereschi, S., Pecchioli, S., Grassotti, A., and Salazar-Orozco, G. (2013). Effects of colored shade netting on the vegetative development and on the photosynthetic activity in several *Hydrangea* genotypes. Acta Hortic. *1000*, 345–352 https://doi.org/10.17660/ActaHortic.2013.1000.47.

Pecchioli, S., Lazzereschi, S., Nesi, B., Antonetti, M., Grassotti, A., and Salazar-Orozco, G. (2013). Morphological characterization of a *Hydrangea* spp. collection. Acta Hortic. *1000*, 99–105 https://doi.org/10.17660/ActaHortic.2013.1000.11.

Schreiber, H.D., Swink, A.M., and Godsey, T.D. (2010). The chemical mechanism for Al^{3+} complexing with delphinidin: a model for the bluing of hydrangea sepals. J. Inorg. Biochem. *104* (*7*), 732–739 https://doi.org/10.1016/j.jinorgbio.2010.03.006. PubMed

Seeram, N.P., Bourquin, L.D., and Nair, M.G. (2001). Degradation products of cyanidin glycosides from tart cherries and their bioactivities. J. Agric. Food Chem. *49* (*10*), 4924–4929 https://doi.org/10.1021/jf0107508. PubMed

Takeda, K., Karıuda, M., and Itoı, H. (1985). Bluening of sepal colour of *Hydrangea macrophylla.* Phytochemistry *24* (*10*), 2251–2254 https://doi.org/10.1016/S0031-9422(00)83019-8.

Yoshida, K., Toyama-Kato, Y., Kameda, K., and Kondo, T. (2003). Sepal color variation of Hydra*ngea macrophylla* and vacuolar pH measured with a proton-selective microelectrode. Plant Cell Physiol. *44* (*3*), 262–268 https://doi.org/10.1093/pcp/pcg033. PubMed