

Colours Intensity and Flower Longevity of Garden Roses

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Abstract: Interactions of garden botanical composition with human senses generate sensations and emotions that may give pleasure and welfare to observers. Among the garden plants, roses are the most important for the wide range of varieties and flower colours. This review is focused on physiological factors that regulate flower longevity and colour persistence in roses that are generally used for garden decorations. Genes that encode for pigments biosynthesis can be used for improving flower colours. The colour persistence of flowers is tightly related to senescence. In fact, senescence associated genes have been discovered and the study of their function may be useful for genetic improvement for extending the flowering periods. The flower colour and the length of the flowering periods are very important factors that contribute to the garden beauty. Colours cannot be objectively appreciated because they can be differently appreciated by the observers. Therefore, sensations and emotions that rose alone or in combinations with other shrubs and perennials in a garden cannot be predictable.

Key words: Colour, enzymes, genes, roses, senescence, vision, Italy

INTRODUCTION

The sight is without doubts, the human sense that receives from gardens the major number of stimuli which give to the observer the impression of masses and spaces, lines and rhythms, shapes and overall colours. Each garden gives to the observer different images that may change from one point to another from one day to another and from one hour to another. Observers may have different perceptions from the same point of view in the same instant from different images such as different are the sensations that they may feel. This means that effects of roses in combination with other garden species are substantially unpredictable. The most important component of this chromatic variability is represented by the garden species and from their reciprocal relationship, from the morphological and physiological states, the development stage and from the phenological stage of each plant (Goodwin, 1988). Plants are able to provide a wide range of colours, from those warm-red, orange and yellow of flowers, fruits and leaves to those cold the green of the foliage, the violet of some fruits and leaves but is the overall of these elements that substantially determines the chromatic effects (Winkel-Shirley, 2001b). Colours from a physics point of view are the result of decomposition of a solar radiation band which corresponds to the visible spectrum (380-760 nm).

Radiations that are involved in the colour spectra are generated by a sequence of signals that starts when light reaches the plants. Part of the radiation is absorbed, transmitted or reflected from the plant pigments. Reflected radiations hit the retina sensors of human eyes and transmit the signals to the observer brain that decodes and elaborates the sensations (Neitz *et al.*, 1996). In simple terms, it is very difficult to associate to garden colours an image pleasant and romantic. However, it is the consciousness that permits to create, keep and enhance the value of chromatic effects which are the main parameters during garden planning, building and management. Seasonality, visibility, pigmentations and flowering durations represent the most important parameters that affect the ornamental values of flowering plants. Plants may have very long vegetative period or quiescence stage which limit their ornamental function or is very modest or even insignificant. Therefore, it is important during garden planning consider the possible interaction of the selected species with other species, in order to guarantee an adequate blooming period that covers the most part of the year. Correct choices of the sowing time, transplanting operations (for seasonal, annual or biannual species), type of pruning (soft or drastic depending on bushes and plants) and the growing cares (tillage, weeds control, irrigations, fertilizations, pathogen controls, etc.) are indispensable for obtaining

an optimal plant combination in a garden. The choice of plants has to be done considering many aspects that are often far from the aesthetical performances but merely based on the economic and logistic point of views. Nevertheless, gardens cannot be without flowering plants or simply without roses.

COLOURS AND PIGMENTS BIOSYNTHESIS

The Union international pour la Protection des Obtentions Vegetales (UPOV) has identified, for varieties registration, 16 groups of colours and each of them is referred to a certain number of cultivars very well known that are used as standard. A new cultivar has to be compared to them for identification. It is an evident simplification for practical use but these groups do not cover the wide range of roses colour. Flower and fruit colours are due to pigments that belong to two classes: flavones and carotenoids (Harborne and Grayer, 1980). Several roses do not have carotenoids or very small amounts. However, γ -ray induced rose mutants showed colour changes compared the wild type with an increase or reduction of carotenoids content (Datta, 1999). Flavonoids include chalcones, aurones, flavonones, isoflavonoids, flavones, flavonols, catechins, leucoanthocyanidins and anthocyanins that are the most wide spread pigments in higher plants. The colour biosynthesis is part of the phenylpropanoids pathway that involves several enzymes such as Phenylalanine Ammonia Lyase (PAL, E.C. 4.3.1.5), Chalcone Synthase (CHS, E.C. 2.3.1.74), Chalcone Isomerase (CHI, E.C. 5.5.1.6), Dihydroflavonol Reductase (DFR, E.C. 1.1.1.219) and Anthocyanidin Synthase (ANS). The ANS is the key enzyme that is involved in anthocyanidins accumulation (Holton and Cornish, 1995; Tanaka *et al.*, 1995; Winkel-Shirley, 2001a) while the last step that leads to anthocyanins synthesis is catalysed by Flavonoid Glycosyltransferases (FGTs). These enzymes regulate the last steps in flavonoid biosynthesis before to transfer them into the vacuole (Vogt and Jones, 2000). Many studies have been demonstrating that the wide range of colours is not only insured by substrates accumulation but also from others factors such as co-pigments, vacuole pH and cell shape (Forkman, 1991; Schiber *et al.*, 2005).

Metal ions, co-pigments (flavons, flavonols) and vacuole pH can affect the colour expression in flowers (Harborne and Grayer, 1980). The increase of pH usually determines blue colour formation in petals that also show up during senescence. The cell shape, mainly the petal cells that accumulate pigments, affects the colour perception exclusively for different optic characteristics. Although, the main factors involved in colour perception

are known, there are many other mechanisms that lead to flower colouration that are not yet understood (Rosati *et al.*, 2003). For example, in some rose cultivars a small amount of blue pigment (rosacyanin) has been found (Fukui *et al.*, 2006). In particularly the cultivar rhapsody in blue accumulates cyanin (cyanidin 3, 5-O-diglucoside) in Anthocyanic Vacuolar Inclusions (AVIs) (Gomnet, 2003).

All species are not able to express the whole colours range for example, roses, carnations and chrysanthemum, which are the most important floricultural crops do not accumulate delphinidin-based anthocyanins. Therefore, violet/blue varieties are not available on the market because they are deficient of Flavonoid 3O, 5O-hydroxylase (F3O5OH), a key enzyme in the biosynthesis of the major blue pigment delphinidin (Nielsen and Podivinsky, 1997; Holton and Tanaka, 1994). Molecular biology tools and plant transformation techniques have made possible to obtain transgenic plants with a modified anthocyanin biosynthetic pathway. A recent review from Tanaka (2006) highlights studies on transgenic plants with pigment pathways altered, including petunia, torenia and carnation by the over expression of heterologous flavonoid biosynthetic genes and/or the down-regulation of endogenous genes.

Rose breeders have long attempted to create blue roses but their efforts have so far only led to pink and pale mauve-coloured flowers. Finally in 2007, the first transgenic blue rose was obtained (Katsumoto *et al.*, 2007). They firstly carried on a preliminary analysis in order to assess the flavonoid composition and the pH of their petal juice of hundreds of rose cultivars, for selecting hosts of genetic transformation that would be suitable for the exclusive accumulation of delphinidin.

The expression of the *viola* F3O5OH gene in some of the selected cultivars resulted in the accumulation of a high percentage of delphinidin up to reach 95% showing a novel bluish flower colour. Then to obtain a dominant, exclusive and independent from the hosts accumulation of delphinidin, a down-regulation of the endogenous Dihydroflavonol 4-Reductase (DFR) gene and an over-expression of DFR gene from the *Iris X hollandica* were performed.

The roses obtained showed delphinidin accumulation exclusively in the petals and the flowers had blue hues not achieved by breeding. Moreover, the ability for exclusive accumulation of delphinidin was inherited by the next generations. The transgenic blue roses obtained from Katsumoto *et al.* (2007) showed for the first time, the delphinidin production. Flowers gained the blue colour achieving a historic milestone in the rose breeding, although more effort will be required to reach the sky blue roses.

COLOURS IN ROSES

The visibility of colours in garden roses is conditioned by genetic, agronomic and environmental factors that are able to give a specific type of flowering. The characteristics of plants and their genome which determines growth and development are obviously the fundamental components that affect the flower colours (Gudin, 2000). The most important components that affect the flowering and the ornamental values in roses are following:

- The habitus of the plant (bush type, climbing, ground-cover roses, dwarf) with the number and type of flowers define the ornamental value of garden roses. However, the branches architecture, the colour and foliage are also important because they may underneath the blooming and flower characteristics
- Repeat-flowering is the ability of plants to produce flowers many times per year. The optimum for garden decoration is to have continuous flowering until the environmental conditions (mainly light and temperature) are in the optimal range (Biran and Halevy, 1974)
- The number of flowers produced at each flowering cycle. This characteristic has been gaining importance for garden roses and the genetic improvement should take in consideration this parameter (Gudin, 2001)
- The number of petals in each flower is another important parameter that is also used for classifying roses as: simple, semi-double and double
- Shape and size of petals, buds and flowers
- Shape, size and colours of hips. The fruits of roses also contribute to prolong the colour intensity and persistence in the garden even if the flowering period is over

All these components but in particular those that are related to colour and flower size are enhanced or repressed by soil exposition, physical and chemical proprieties. In particular, the pH and calcium directly affect flower longevity and pigments (Kondo *et al.*, 2005; Torre *et al.*, 1999). The soil salinity does not have specific action on flower colouration but leaves and flowers may show colour alterations if salt concentration overcomes the tolerance thresholds specific of the cultivar.

Agronomic factors also include all the technologies that allow plants to express their genetic potential (in terms of development, growth and differentiation) (Gudin, 2001). Growing techniques, fertilization, irrigation, pruning and eventual flower harvesting directly or indirectly affect the flower colouration (Harborne and Grayer, 1980). Among them the most important is the

pruning. This practice has been always considered a sort of taboo performed on the basis of certain rules often without any scientific basis. A correct rose pruning has to be performed by observing the plant in the different development stages. The effect of the previous pruning may give important tips for the next one. The good sense is often enough for performing a rational pruning on garden roses. The objectives of a good pruning are to guarantee a good health status of plants, shape and flowering. The type of pruning affects the rose flowering performances, therefore it is better to underline the following points:

- A pruning too long induces an earlier flowering but small size of flowers and alters the vegetative equilibrium inducing vegetation on the top of the plant
- A pruning too short delays the emission of shoots but they will be vigorous with few flowers. In some cases, the plant may not produce any flowers
- An adequate pruning should be performed considering the ratio between the length and diameter of the branch that should be pruned. However, never consider only the pruning length that may lead to a wrong choice
- Do not allow producing fruits on weak plants, otherwise they will become weaker

On the basis of the above suggestions, it is necessary to leave on the plant a high density of vegetation, especially if they are young plants in active growth for guarantee a good photosynthesis.

Among environmental conditions, light and temperature are the most important on the flowering process. Flower development is directly correlated with solar radiation as shown by growth models used for predicting the cut flower production in greenhouse. The air Relative Humidity (RH) instead mainly affects the flower longevity rather than flowering (Plaut *et al.*, 1979). Moreover, the RH increases the sensitivity to botrytis and deeply influences the calcium accumulation in the petal cells with subsequently effects on senescence. The colour stability has been studied in modern garden roses. The most part of this study has been performed on Hungarian roses. Results showed that Hungarian bred floribundas had a lower colour changes than the polyanthas or climbing roses (Boronkay *et al.*, 2009).

EFFECT OF TEMPERATURE AND LIGHT ON ANTHOCYANINS BIOSYNTHESIS

The temperature greatly affects the anthocyanins biosynthesis. Roses produced under cooler environments

have higher anthocyanins content especially during summer period (Plaut *et al.*, 1979). High temperatures instead, such as 39°C, applied at different stage of flower development reduce the anthocyanins content in petals (Dela *et al.*, 2003). Analogous results were found in chrysanthemum petals (Huh *et al.*, 2008). Pigment losses are higher in environment conditions characterised by High Temperature and Low Light conditions (HTLL). The pigment reduction in HTLL conditions is a result of anthocyanins breakdown and down-regulation of genes encoding for enzymes involved in the biosynthesis (Gonzalez, 2009). *In vitro* cultivation of petals exposed to different light sources demonstrated that UV-B radiations for 12-18 h induced phenylpropanoid genes expression and anthocyanins accumulation (Hennayake *et al.*, 2006).

COLOURS PERSISTENCE AND FLOWER SENEESCENCE

The decorative effect of roses depends from the duration of the flowering period that is regulated by many factors such as cultivars, environment and growing management. Species and cultivars have a specific genetic background that affects the time of flowering, type of flowering, type of flower, repeat-flowering, resistance and tolerance to abiotic stresses such as low temperatures etc. In addition to these factors which are related to the period and the continuity of flower production, there are others that are correlated with flower longevity. These parameters are directly correlated with the ornamental beauty of roses.

Flower life is regulate by the senescence process and usually ends with petals abscission. Flower senescence in roses depends by plant hormones such as ethylene, Abscisic Acid (ABA) and Calcium (Ca). Little information is available about flower senescence of garden roses which can be ascribed to natural senescence. On the contrary many research works have been publishing on flower senescence of cut or potted roses (Serek and Andersen, 1993; Muller *et al.*, 2000).

Ethylene is a plant hormone that is involved in many physiological and biochemical processes in higher plants such as germination, growth, development, ripening and senescence (Chen *et al.*, 2005). Its effect on plant is regulated by tissue sensitivity. Plant sensibility to ethylene is extremely variable. Roses are very sensitive to ethylene which reduces their ornamental value. The ethylene sensitivity in roses varies within the same species and from cultivars to cultivars (Van Doorn, 2002). A high ethylene production is not always associated to a shorter flower life or faster senescence. These evidences have been proved by treatments with ethylene

biosynthesis inhibitors such as Amino-Oxyacetic Acid (AOA) which delays flower senescence of victory parade potted roses (Serek and Andersen, 1993). Analogous results were obtained using Silver Thiosulphate (STS), an ethylene action inhibitor. This inhibitor is more efficient of ethylene biosynthesis inhibitors such as AOA (Mensuali-Sodi *et al.*, 2005). Moreover, in rose but also in petunia treatments with AOA induced petal discolouration (Ferrante *et al.*, 2006).

Plant sensitivity to ethylene depends from the presence of receptors that regulate the physiological responses. The mechanism that regulates the ethylene receptors biosynthesis is not completely clear. Roses ethylene sensitivity increases by increasing the receptor expression as it has been demonstrated in two potted rose cultivars (Bronze and Vanilla) which had different ethylene sensitivity (Muller *et al.*, 2000). Treatments with exogenous plant hormones demonstrated that some of them are activated by ABA. This hormone seems to have, in this species an important role during flower senescence (Le Page-Degivry *et al.*, 1991). Exogenous applications of ABA increased the percentage of flower and leaf abscission in potted roses, without increasing ethylene production.

Moreover, ethylene action inhibitors did not prevent ABA effect. These results demonstrated that ABA induces flower senescence in rose independently by the ethylene presence. During flower development endogenous ABA decreases from bud to fully open stage and increases during senescence (Muller *et al.*, 1999). However this trend such as the ethylene sensitivity varies among cultivars. Flowers with higher ABA content have also higher ethylene sensitivity with higher ethylene receptor expression (Muller and Stummann, 2003).

Flowers grown in high RH conditions have longer life with lower Ca content. Translocation of Ca in plants occurs through the xylem pathway. Hence, plant organs that have lower transpiration rate such as flowers may show deficiency symptoms. The Ca concentration in petals regulates the membrane permeability and functionality. It also reduces the ethylene production, protein and phospholipids degradation. High Ca concentration in petals slows down the senescence process and delays the turgor losses insuring the petal cells vitality (Torre *et al.*, 1999). The effect of Ca is specific and cannot be replaced by other cations. High levels of calcium in tissues and in the neo-formed petals reduce the botrytis and decolouration of petal margins, two symptoms associate to the senescence process.

Garden roses have to display as long as possible their colours. Therefore, the senescence process should be delayed. The flower life can be easily extended using roses that are not sensitive to ethylene or produce very

low amounts of this plant hormone. The classic genetic improvement in several crops was oriented to select plants that do not produce ethylene. Unfortunately, the results of this research brought to select plants that do not have perfume. Some breeding companies are going back to restore the scent biosynthesis in roses using old cultivars.

HUMAN PERCEPTION AND ROSE COLOURS

Colour perception is a peculiarity tightly personal and depends from two factors which are correlated to the physics nature and to the personal sensitivity that may give specific sensations or emotions. However, the colour visual defects of human eyes such as dichromatic (dichromats have complete deficiency in one cone pigment but preserves the remaining two cone pigments), monochromatic (person who has only one cone pigment), achromatopic (possesses no functioning cones) can alter the judgment about the beauty of a garden (Neitz *et al.*, 1996). Different observers may have a different vision of the same object. Therefore, each individual has an own vision system and specific emotive sensitivity towards colours. It is difficult to plan a determinate colours combination (plant combinations) because what is imagined during planning may be different when a garden is realized.

The objective ability of observer to percept colours, in this case for flowers, depends from a wide number of factors such as vegetative habitus, growing shape (bush, climbing, dwarf, small tree), soil exposition, relationship among structural elements and plants. Moreover, in the garden, relationship among the different rose cultivars, the soil physics and chemical characteristics, growing techniques, fertilizations, irrigations, pruning and eventual flower harvesting, growth-development stage, the observation point and the emotion state of the observer, the last but the most important is the plant genotype that represents the potential ability to express colours. The large number of components can give infinite colour combinations in a garden.

CONCLUSION

The rose has an important role as ornamental plant and has absolute prominence among the flowering plants of a garden. Roses are also very important as potted plants and have a primary role as cut flowers. In according to some researchers the rose is the first cultivated ornamental plant. The genetic improvement produced a wide range of varieties that are able to satisfy almost all needs. However, all research done in the genetic improvement in this genus has been carried out using only 11 species out of 200 available. Hence, there is a wide

range of natural genetic sources that can be used for improving the colour and the flower life of garden roses using both classic and biotechnological genetic improvement.

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REFERENCES

- Biran, I. and A.H. Halevy, 1974. Effect of short-term heat and shade treatments on petal colour of 'Baccara' roses. *Physiologia Plantarum*, 31: 180-185.
- Boronkay, G., E. Jambor-Benczur and A. Mathe, 2009. Colour stability of the flowers of some rose varieties measured in CIEDE2000. *Hortic. Sci.*, 36: 61-68.
- Chen, Y.F., N. Etheridge and G.E. Schaller, 2005. Ethylene signal transduction. *Ann. Bot.*, 95: 901-915.
- Datta, S.K., 1999. Flower colour analysis in garden roses: Carotenoids. *Sci. Hortic.*, 6: 151-156.
- Dela, G., E. Or, R. Ovadia, A. Nissim-Levi, D. Weiss and M. Oren-Shamir, 2003. Changes in anthocyanin concentration and composition in 'Jaguar' rose flowers due to transient high-temperature conditions. *Plant Sci.*, 164: 333-340.
- Ferrante, A., P. Vernieri, F. Tognoni and G. Serra, 2006. Changes in abscisic acid during floral senescence. *Biologia Plantarum*, 50: 581-585.
- Forkman, G., 1991. Flavonoid as flower pigments: The function of the natural spectrum and its extension by genetics engineering. *Plant Breed.*, 106: 1-26.
- Fukui, Y., K. Nomoto, T. Iwashita, K. Masuda, Y. Tanaka and T. Kusumi, 2006. Two novel blue pigments with ellagitannin moiety, rosacyanins A1 and A2, isolated from the petals of *Rosa hybrida*. *Tetrahedron*, 62: 9661-9670.
- Gonnet, J.F., 2003. Origin of the color of cv. Rhapsody in Blue rose and some other so-called 'Blue' roses. *J. Agric. Food Chem.*, 51: 4990-4994.
- Gonzalez, A., 2009. Pigment loss in response to the environment: A new role for the WD/bHLH/MYB anthocyanin regulatory complex. *New Phytologist*, 182: 1-3.
- Goodwin, T.W., 1988. *Plant Pigments*. Academic Press Ltd., London.
- Gudin, S., 2000. Rose: Genetics and breeding. *Plant Breed. Rev.*, 17: 159-189.
- Gudin, S., 2001. Rose breeding technologies. *Acta Hortic.*, 547: 23-26.

- Harborne, J.B. and R.J. Grayer, 1980. The Anthocyanins. In: The Flavonoids, Advances in Research Since 1980, Harborne, J.B. (Ed.). Chapman and Hall, London, pp: 1-20.
- Hennayake, C.K., M. Kanechi, N. Yasuda, Y. Uno and N. Inagaki, 2006. Irradiation of UV-B induces biosynthesis of anthocyanins in flower petals of rose, *Rosa hybrida* cv. 'Charleston' and 'Ehigasa'. *Environ. Control Biol.*, 44: 103-110.
- Holton, T.A. and E.C. Cornish, 1995. Genetic and biochemistry of anthocyanin biosynthesis. *Plant Cell*, 7: 1071-1083.
- Holton, T.A. and Y. Tanaka, 1994. Blue roses-a pigment of our imagination?. *Trends Biotechnol.*, 12: 40-42.
- Huh, E.J., H.K. Shin, S.Y. Choi, O.G. Kwon and Y.R. Lee, 2008. Thermosusceptible developmental stage in anthocyanin accumulation and color Response to high temperature in red chrysanthemum cultivars. *Korean J. Hortic. Sci. Technol.*, 26: 357-361.
- Katsumoto, Y., M. Fukuchi-Mizutani, Y. Fukui, F. Brugliera and T.A. Holton *et al.*, 2007. Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. *Plant Cell Physiol.*, 48: 1589-1600.
- Kondo, T., T.K. Yuki and K. Yoshida, 2005. Essential structure of co-pigment for blue sepal-color development of hydrangea. *Tetrahedron Lett.*, 46: 6645-6649.
- Le Page-Degivry, M.T.H., M. Orlandini, G. Garelo, P.H. Barthe and S. Gudin, 1991. Regulation of ABA levels in senescing petals of rose flowers. *J. Plant Growth Regul.*, 10: 67-72.
- Mensuali-Sodi, A., A. Ferrante, F. Tognoni and G. Serra, 2005. Inhibitors of ethylene action and biosynthesis on cut carnation. *Agricoltura Mediterranea*, 135: 55-58.
- Muller, R. and B.M. Stummam, 2003. Genetic regulation of ethylene perception and signal transduction related to flower senescence. *J. Food Agric. Environ.*, 1: 87-94.
- Muller, R., B.M. Stummam, A.S. Andersen and M. Serek, 1999. Involvement of ABA in postharvest life of miniature potted roses. *Plant Growth Regul.*, 29: 143-150.
- Muller, R., B.M. Stummam and M. Serek, 2000. Characterization of an ethylene receptor family with differential expression in rose (*Rosa hybrida* L.) flowers. *Plant Cell Rep.*, 19: 1232-1239.
- Neitz, J., M. Neitz and P.M. Kainz, 1996. Visual pigment gene structure and the severity of color vision defects. *Science*, 274: 801-804.
- Nielsen, K.M. and E. Podivinsky, 1997. cDNA cloning and endogenous expression of a flavonoid 3050-hydroxylase from petals of lisianthus (*Eustoma grandiflorum*). *Plant Sci.*, 129: 167-174.
- Plaut, Z., N. Zieslin, A. Grawa and M. Gazit, 1979. The response of rose plants to evaporative cooling: Flower production and quality. *Scientia Horticulturae*, 11: 183-190.
- Rosati, C., P. Simoneau, D. Treutter, P. Poupard, Y. Cadot, A. Cadic and M. Duron, 2003. Engineering of flower color in forsythia by expression of two independently-transformed dihydroflavonol 4-reductase and anthocyanidin synthase genes of flavonoid pathway. *Mol. Breed.*, 12: 197-208.
- Schiber, A., K. Mihalev, N. Berardini, P. Mollov and R. Carle, 2005. Flavonol glycosides from distilled petals of *Rosa damascena* Mill. *Z Naturforsch.*, 60: 379-384.
- Serek, M. and A.S. Andersen, 1993. AOA and BA influence on floral development and longevity of potted Victory Parade miniature rose. *HortScience*, 28: 1039-1040.
- Tanaka, Y., 2006. Flower colour and cytochromes P450. *Phytochem. Rev.*, 5: 283-291.
- Tanaka, Y., Y. Fukui, M. Fukuchi-Mizutani, T.A. Holton, E. Higgins and T. Kusumi, 1995. Molecular cloning and characterization of *Rosa hybrida* dihydroflavonol 4-reductase gene. *Plant Cell Physiol.*, 36: 1023-1031.
- Torre, S., A. Borochoy and A.H. Halevy, 1999. Calcium regulation of senescence in rose petals. *Physiologia Plantarum*, 107: 214-219.
- Van Doorn, W.G., 2002. Effect of ethylene on flower abscission: A survey. *Ann. Bot.*, 89: 689-693.
- Vogt, T. and P. Jones, 2000. Glycosyltransferases in plant natural product synthesis: Characterization of supergene family. *Trends Plant Sci.*, 5: 380-386.
- Winkel-Shirley, B., 2001a. Flavonoid biosynthesis: A colourful model for genetics, biochemistry, cell biology and biotechnology. *Plant Physiol.*, 126: 485-493.
- Winkel-Shirley, B., 2001b. It takes a garden: How work on diverse plant species has contributed to an understanding of flavonoid metabolism?. *Plant Physiol.*, 127: 1399-1404.