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Rose: Some Important Findings with Special Reference to Physiology of Flowering

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ABSTRACT

Rose, a commercial crop, is distributed worldwide. Due to several natural and artificial changes over time the *Rosa* species have become very difficult to determine. However, successful attempts have been made to classify the genus *Rosa* with the aid of cytological and phenotypic studies. The crop also encounters serious problems of biotic stress and issues related to flower production and enhancement of their shelf-life. These problems are addressed with difficulty through conventional breeding and biotechnological approaches are currently playing a vital role in the rose improvement programme. On the other hand, biochemical and physiological studies are powerful approaches toward understanding organ differentiation and their growth and development. However, relatively little is known about the physiology of flowering in rose. The whole natural period of growth and development is much shorter for petals than leaves and hence petals seem to be an excellent model system for the study of fundamental physiological processes. The present chapter extensively summarizes the studies conducted on various physiological and biochemical aspects of flowering with special reference to two species of scented rose i.e. *Rosa damascena* and *R. bourboniana*. Such a review will be helpful to expand our insight of the flowering behaviour and in finding out suitable indicators of various physiological processes which may help in enhancing essential oil production of both the diverse species of rose.

Keywords: *in vitro* propagation, growth and development, organ differentiation, *Rosa damascena*, *Rosa bourboniana*, scented rose

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INTRODUCTION

Rose, being one of the most important commercial crops is associated with many problems. The infestation by pests, fungal (black spot, powdery mildew, die back etc.) and viral diseases cause huge revenue losses to the crop. Cut flower roses are susceptible to vascular blockages that cause premature wilting (reviewed in Balas *et al*. 2006). Furthermore, increase of vigour, flowering duration and post-harvest shelf life are some of the major challenges in crop production. *Rosa damascena* Mill (Damask rose), commonly known as scented rose, is an important essential oil yielding crop (Younis *et al*. 2007). High valued rose oil (0.045%) obtained from the flowers on distillation, is used in high grade perfumery and cosmetic industries. The total flowering period in a year in this species is only around 25-30 days during April-May. *Rosa bourboniana* Desport (Edward rose), also an essential oil (0.015%)-yielding crop, is popular on account of its long blooming period, ease of propagation and fragrance of its flower. Since the oil content in this species is much less, it is thus less productive (Anonymous 1972). The flowering in this species is sporadic and it flowers three times a year i.e. April-May, July-August and mid October. However, April-May is the only main flowering period in both these species. There is an urgent need to combine expanded flowering time with oil content. In the above context, production of novel cultivars with desirable traits is of urgent need in a rose improvement programme. Traditionally, rose improvement involved sexual crossing and selection, together with the identification of natural

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Invited Review

mutations. Although, desirable traits have been introduced by conventional breeding methods, certain constraints still remain: (1) a narrow genetic base, only few species of *Rosa* are used for breeding purposes; (2) distant crosses are limited by incompatibility or differences in ploidy level between putative parents; (3) conventional breeding methods are slow due to the perennial nature of the crop; (4) a high degree of sterility caused by discordant chromosome numbers, and (5) the delicate balance of factors determining plant growth and development were altered by sexual crossings and selection (Mol *et al*. 1989). In the present article we have discussed in detail various aspects such as taxonomy, *in vitro* propagation, physiological and biochemical studies, of two important species of rose viz. *Rosa damascena* Mill and *Rosa bourboniana* Desport. Readers are recommended to read more comprehensive reviews on the biotechnology of rose (Khosh-Khui and Teixeira da Silva 2006; Pati *et al*. 2006).

HISTORY AND ORIGIN OF ROSE

Fossils of rose, found in Oregon and Colorado (USA) are estimated to be more than thirty million years old (Fairbrother 1965). There is also evidence that roses were cultivated 5,000 years ago by the ancient civilizations of China, western Asia, and northern Africa (Shepherd 1954). Moreover, in antiquity these were used to decorate the tombs of the Greek and Chinese leaders. Roses symbolized secrecy in Rome, virtue in the Far East, and silence in Egypt (Rowley 1966). Considerable information about roses in antiquity is also found in the writings of the Greek historian Herodotus (490-420 BC) and the Greek philosopher Theophrastus (372-287 BC).

Ancestors of modern roses are the wild roses. Most of the cultivated roses are found in the temperate zones of the northern hemisphere, and Asia seems to be the gene center where the majority of species are found (Broertjes and van Harten 1978). Eleven species namely, *Rosa brunonii*, *R. eglanteria*, *R. foetida*, *R. gigantea*, *R. involucrata*, *R. leschenaultiana*, *R. longicuspis*, *R. microphylla*, *R. moschata*, *R. rubiginosa* and *R. sericea* are purportedly growing wild in India.

ECONOMIC IMPORTANCE OF ROSE

More than 20 million rose bushes are planted each year in gardens the world over. Importance of roses as a cut flower is indicated by sale of more than 4 billion blooms with an approximate annual retail value of US$ 11 billion. Worldwide, 4000 ha were devoted to greenhouse production of 6 billion cut flowers in 1990, and the production has subsequently increased (Pertwee 1995). Consumption of cut flower rose stems alone amounted to 45 billion yen in Japan, the world's greatest cut-flower consuming nation, in 2004 (Xia *et al*. 2006). Further, greenhouse production areas worldwide for cut rose have increased by more than 5,250 ha in 1994 (cf. Gudin 2000). In addition, millions of miniature potted roses mainly produced in northern Europe and north America, are sold every year (Borch *et al*. 1996).

The use of rose in the perfume industry as well as its medicinal and culinary qualities are well documented (Shepherd 1954). Amongst scented roses, *Rosa damascena* Mill., occupies the most important position for the extraction of essential oil. It is cultivated in Bulgaria, France, Italy, Turkey, Iran, Morocco and U.S.A for the production of attar (otto) of rose or oil of roses (Krussmann 1981). Attar is widely used in the cosmetic industry and is one of the most highly prized essential oils (Lata and Gupta 1971). The estimated wholesale price of attar is US$ 4,500 per Kg (http://www.adventurearabia.com/arabianaromasnf.htm).
The other products from *R. damascena* and other scented species of rose are rose water, rose concrete, rose absolute and "gulkand" (Anonymous 1972; Younis *et al*. 2007).

BOTANICAL DISTRIBUTION, TAXONOMY, CHROMOSOME NUMBER

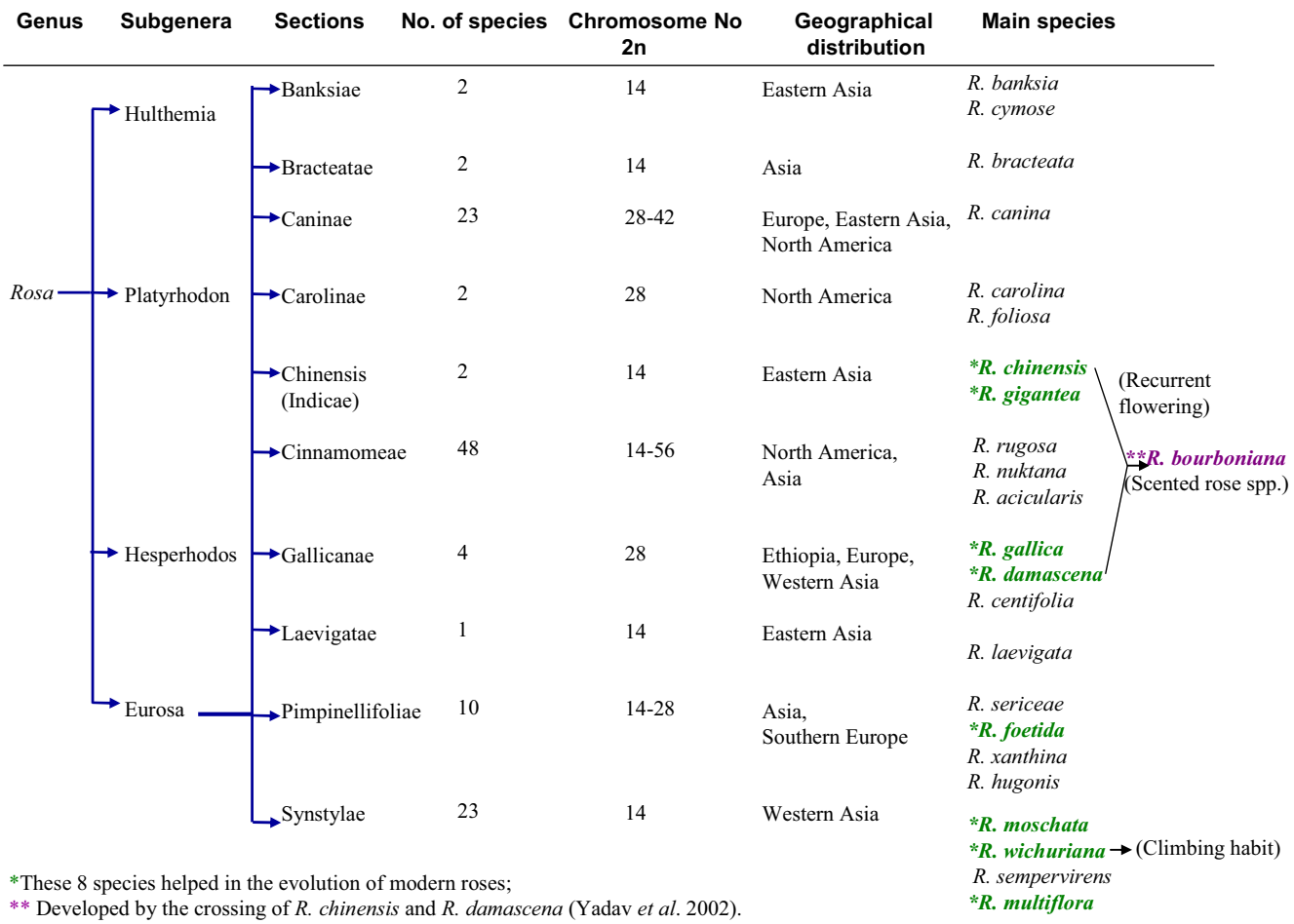
There are about 120 species of roses which are found throughout the temperate regions of the northern hemisphere, from Arctic Circle to the subtropics (Marshall 1973; Maia and Venard 1976; Zieslin and Moe 1985), including USA (New Mexico), Iraq (Marshall 1973), Ethiopia, Bengal, and southern China (Zieslin and Moe 1985).

Taxonomy

The genus *Rosa* belongs to the family Rosaceae and sub family Rosoideae. Shepherd (1954), in his paper has quoted Linnaeus, 1753, who while classifying roses remarked that "the species of *Rosa* are very difficult to determine, and those who have seen a few species can distinguish them more easily than those who have examined many". This is due to several changes, both natural (primarily crossing in nature and mutations) and artificial (hybridization) over a period of time. It is almost impossible to distinguish between pure species, hybrids, garden forms with Latinized names, and cultivars, many of which are synonyms (Zieslin and Moe 1985).

The genus *Rosa* is divided into 4 subgenera, namely, Hulthemia, Platyrhodon, Hesperhodos and Eurosa (**Fig. 1**; Rehder 1940; Darlington and Wylie 1955; Maia and Venard 1976). Eurosa is a major donor associated with the origin of modern roses. The subgenus Eurosa is further classified into 10 sections, of which 7 sections possess adnate stipules and are more significant as far as garden roses are concerned. Out of these seven, two sections, Indicae and Synstylae, are distinguished from the other five by their exserted styles, which are free in the former and fused in the latter. These two sections contain species of importance to garden rose development. The short styled sections include Pimpinellifoliae, which contains *R. foetida*, an important ancestor of garden rose. The section Gallicanae contains important ancestral species like *R. gallica* and its hybrids, namely *R. damascena* and *R. centifolia*. The largest section Cinnamomeae (48 species) contains *R. rugosa*, *R. acicularis* and *R. nuktana*, which have played only a minor role in the development of modern roses. More recently molecular markers have been extensively used to discriminate rose species (see review by Yan *et al*. 2006).

Out of the 120 or more wild species of the genus *Rosa*, only eight species viz. *R. chinensis*, *R. damascena*, *R. foetida*, *R. gallica*, *R. gigantea*, *R. moschata*, *R. multiflora*, and *R. wichuriana* of the sections Indicae, Gallicanae, Pimpinellifoliae and Synstylae are said to have played an important role in the evolution of modern garden roses (Anonymous 1972). A schematic representation of evolution of modern garden roses is presented in **Fig. 2**. Until the beginning of the 19th century, most of the cultivated European roses that flowered mostly in summer were derived from *R. gallica* and *R. damascena*. About this time, a few cultivated types of roses derived from *R. chinensis* and *R. gigantea* were introduced from the Far East. They were perpetual flowering types, and some of them were characterized by a sweet scent reminiscent of tea, and others, by yellow flowers. The Noisette group of roses was the earliest garden roses developed by crossing *R. moschata*, the Musk rose with *R. chinensis*. They bear flowers in large bunches and are perpetual flowering types. The Noisettes were later back crossed with a yellow flowered Chinese type to give rise to Yellow Teas. The hybrid china and bourbon rose groups were the other early derivatives obtained by crossing the Chinese type with the type derived from *R. gallica* and *R. damascena*, respectively. By crossing these hybrids again with each other, the well known Hybrid Perpetual roses were obtained. Bourbon was backcrossed with one of the pink flowered, tea scented Chinese introductions to give rise to Pink Teas. The Hybrid Perpetuals were in turn crossed with derivatives of the Pink and Yellow Teas to give rise to the well known Hybrid Tea roses. These are charac-



*These 8 species helped in the evolution of modern roses;
** Developed by the crossing of *R. chinensis* and *R. damascena* (Yadav *et al*. 2002).

Fig. 1 Classification of the genus Rosa. (Based on: Yadav *et al*. 2002).

terized by their hardiness and continuous flowering. They were the dominant garden roses for quite a long time (Anonymous 1972).

In the early part of the 20th century, dwarf Polyanthas were derived by crossing *R. multiflora* with dwarf *R. chinensis*. The hybrids were then crossed with Hybrid Tea types to give rise to the initial group of Hybrid Polyantha roses. The Hybrid Polyanthas were backcrossed to Hybrid Teas in the evolution of modern Floribundas that constitute the dominant garden roses today. Other significant developments in breeding garden roses are the use of *R. foetida*, that has enriched the available range of colours by introducing deep yellow and related combinations, and *R. wichuriana*, which has introduced the climbing habit (Anonymous 1972).

A classification of scented roses used for the extraction of perfumery products is also available (Padhye 1982). Primarily four species are used for extraction of rose attar (or oil) namely *R. damascena*, *R. centifolia*, *R. alba* and *R. gallica* (Singh and Malik 1982). Amongst these, *R. damascena*, the predominantly grown species for essential oil, the summer Damask rose is suspected to have arisen from *R. gallica* × *R. phoenicia*, while autumn Damask is a hybrid of *R. gallica* × *R. moschata* (Hurst 1941). In India, only two main species, *R. damascena* and *R. bourboniana*, are commercially cultivated as scented rose (Pal 1972). *R. bourbonniana* is a natural hybrid between Parson's Pink China rose (*R. chinensis*) and autumn Damask rose (*R. damascena bifera*) (Yadav *et al*. 2002).

Cytological studies

Cytological studies contribute substantially to rose taxonomy. Chromosome numbers in the genus *Rosa* are based on multiples of seven and range from 2n=14=2x to 2n=56=8x (Darlington and Wylie 1955). Aneuploids are rare (Rowley 1960). The subgenera, Hulthemia, Platyrhodon and Hesperodos, have only one species each, in which 2n=14=2x. The fourth subgenus, Eurosa, contains over 120 species grouped into ten sections (Rehder 1960). In sections Banksianae, Bracteatae, Indicae, Lavigatae and Synstylae 2n=2x, in the Gallicanae 2n=4x, in the Carolinae and Pimpinellifoliae 2n=2x and 4x, in the Caninae 2n=4x, 5x and 6x, and in the Cinnamomeae 2n=2x, 4x, 6x and 8x (Darlington and Wylie 1955).

Botany

The rose is an ornamental shrub with upright or climbing stems, usually prickly. The leaves are alternate, compound, pinnate (*R. persica* has undivided leaves), with stipules adherent to the leaf stalks. Flowers are solitary or in corymbs. Calyx is five lobed, lobes either simple or compound, and inserted at the top of a roundish or pear shaped fleshy tube. Petals and sepals are generally five (*R. sericea* has only four) but many more in some cultivars due to modification of stamens. Below petals, it has a deep hypanthium (**Fig. 3**; www.backyardnature.net). Inside the hypanthium are many free carpels (apocarpous gynoecium). Each carpel has a superior ovary enclosed by the hypanthium. The styles protrude through the opening in the top of the hypanthium. The fruit of *Rosa* is a "hip" formed by the fleshy hypanthium. Inside the hip are many achenes. An achene is the basic fruit type in subfamily Rosoideae.

PROPAGATION

Vegetative propagation

Roses are propagated both by seeds and other vegetative

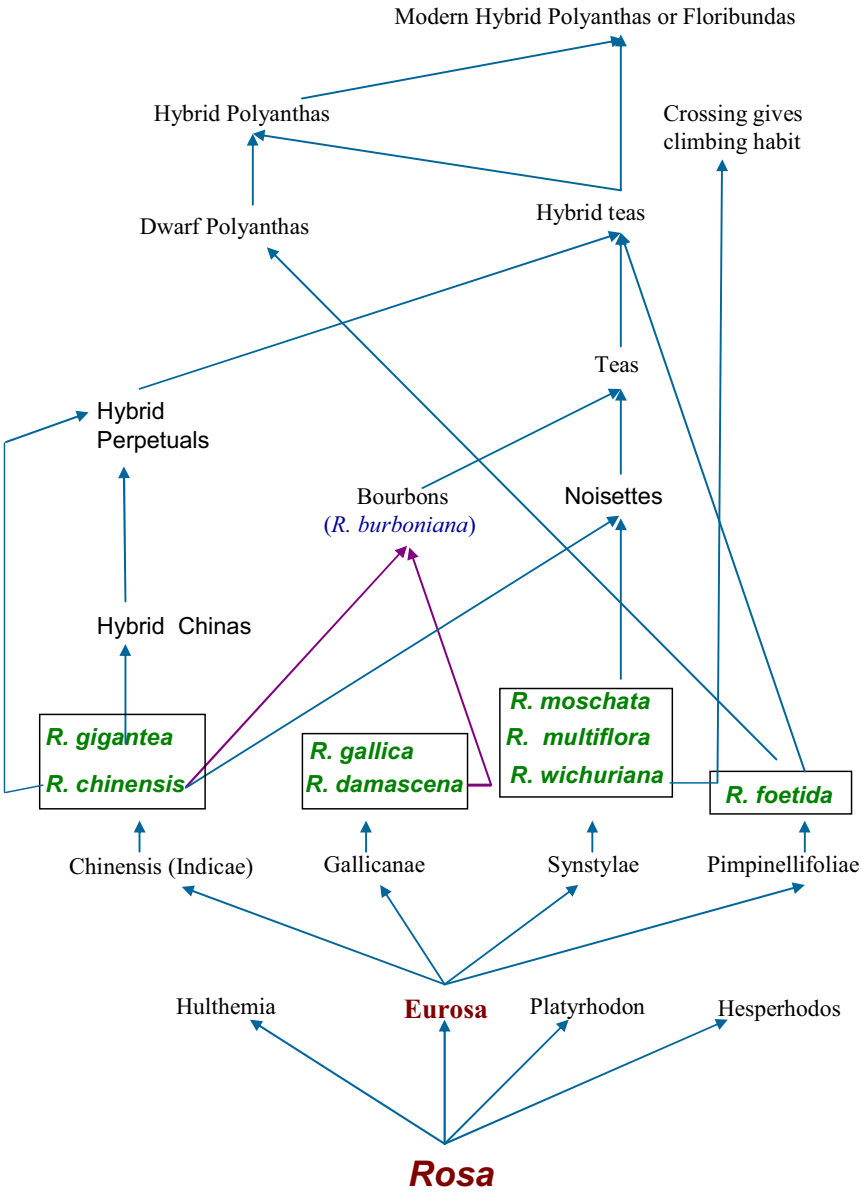


Fig. 2 Evolution of modern garden roses. (Source: Anonymous 1972).

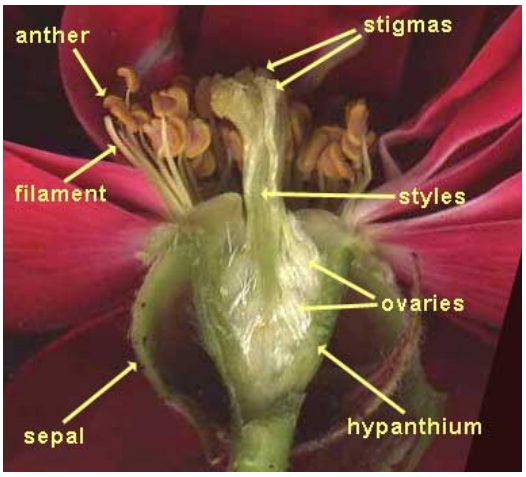


Fig. 3 Structure of rose flower. (With kind permission, Jim Conard; www.backyardnature.net).

methods like cutting, layering, budding and grafting (Yadav *et al*. 2002). Seeds, obtained from mature fruits, are generally used by breeders for development of new cultivars. Seedlings are also used as stock for grafting and budding. Rose seedlings tend to flower even when very young and then further growth is hampered. Therefore, in order to get large and sturdy seedlings, it is desirable to pinch off the buds at a very early stage.

Propagation by cutting is also done to raise stocks for grafting. Cutting may be stem or root cuttings. The quality of the plant is influenced by the time of the year when cuttings are made. Monsoon or spring are the most appropriate seasons for stem cuttings. Cuttings may be single, double or triple eyed. The latter one is generally preferred as there are greater chances of getting more shoots and better root formation. Factors influencing regeneration of rose plants from cuttings are type of cuttings, treatment of cuttings and environmental conditions during rooting. The age and physiological condition of plant exert a strong influence on the development of roots. The cuttings taken from 5 year old plants rooted better than those taken from 20 year old plants. Hard wood cuttings are widely used in the propagation of rose root stocks. In case of root cuttings, young and healthy plants should be chosen. The most important aspect to be taken into consideration is polarity when planting. Climbing and rambling roses are usually propagated by layering. This technique includes air-layering and ground-layering. Other methods used for vegetative propagation are grafting and budding.

In vitro propagation

Although conventional propagation is a predominant tech-

nique in roses, yet it does not ensure healthy and disease-free plants. Moreover, dependence on season and slow multiplication rates are the other major limiting factors. In the last few years, *in vitro* propagation has revolutionized commercial nursery business (Teixeira da Silva 2006a). Significant features of *in vitro* propagation procedure are its enormous multiplicative capacity in a relatively short span of time; production of healthy and disease free plants; and its ability to generate propagules around the year (Dhawan and Bhojwani 1986). Using this technology, up to 400,000 plants could be cloned, from a single rose on an annual basis (Martin 1985). Such a method has considerable implications for the rose breeder as it allows rapid multiplication of new varieties. Micropropagated plants are well suited for cut flower production as they are more compact (Onesto *et al*. 1985), branch better and sometimes yield more flowers (Reist 1985). In addition, tissue culture-derived dwarf roses used for pot plant production have a faster rate of growth, early flowering, and exhibit shorter shoots and more laterals than conventionally produced plants (Dubois *et al*. 1988). The history of rose tissue culture dates back to 1945, when Nobecourt and Kofler succeeded in obtaining callus and roots on the explanted buds. Lammerts (1946) for the first time reported the use of embryo culture in rose breeding. Studies were initiated by Nickell and Tulecke (1959) and Weinstein *et al*. (1962) to culture cells, cell suspension and calli with a view to understand differentiation and regeneration. The first shoot organogenesis from callus tissue was reported by Hill (1967) in a climbing Hybrid Tea rose 'The Doctor'. The earliest references of rose micropropagation were those of Jacob *et al*. (1969, 1970a, 1970b) and Elliott (1970) in *R. hybrida* cv. 'Superstar' and *R. multiflora*, respectively. Since these pioneering efforts, a lot of data were generated and a number of papers have been published on different aspects of *in vitro* studies of rose with a greater emphasis on micropropagation. These details are covered with much more depth elsewhere (Khosh-Khui and Teixeira da Silva 2006; Pati *et al*. 2006).

A successful micropropagation protocol proceeds through a series of stages, each with a specific set of requirements. These are (i) initiation of aseptic cultures, (ii) shoot multiplication, (iii) rooting of microshoots, and (iv) hardening and field transfer of tissue culture raised plants. In rose improvement programme, rapid multiplication of elite clones, production of healthy and disease-free plants and faster introduction of novel cultivars with desirable traits are of urgent need. At present, there are many reproducible protocols for *in vitro* propagation of rose. However, the new challenges that are faced today by the tissue culture industry include cost efficiency, automation, control and optimization of the microenvironment, etc. It is, therefore, important to bring about further improvements in the existing tissue culture protocols. The recent trend in shift of the status of the medium from agar-gelled to liquid medium is a strategic step in this direction. The effective use of liquid medium during shoot multiplication and in rooting is a cost effective proposition and a step towards automation and commercialization. *In vitro* propagation of rose via somatic embryogenesis offers a great potential for rapid propagation and improvement, and direct regeneration protocols using leaf explants from *in vitro* raised shoots could be effectively used in maintaining the clonal fidelity of elites and in genetic transformation programmes.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES

Flowering, often associated with senescence and death, is a dramatic phenomenon in monocarpic plants, where the whole plant dies after flowering or fruiting. In polycarpic plants, death is restricted to parts of the flower itself- those which senesce and abscise soon after flowering. This process represents a dramatic change in the pattern of differentiation at the shoot apex or in the axillary buds close to it. Such a complex process is regulated by both internal factors and environmental signals. In most cases, the flower is the organ with the shortest period of longevity (Haley and Mayak 1981), which varies greatly from species to species, but is relatively constant within any one species. The control mechanisms of flower growth and development during its life span have been reported only in few cases (Eason and Webster 1995; reviewed in detail in Teixeira da Silva 2006b). Petals are the floral organs that primarily determine the commercial longevity of flowers, and as a consequence much attention has been given to the physiological, biochemical and genetic processes that occur during petal development (Eason and Webster 1995). This developmental process is characterized by several phases including differentiation, cell division, cell enlargement, and ultimate death i.e. senescence. Much of the petal growth is associated with the result of cell enlargement. Relatively little is known on the physiology of flower and petal in comparison with that of other plant organs, such as leaves. The whole natural period of growth and development is much shorter in petals than in leaves and hence petals seem to be an excellent model system for the study of fundamental physiological processes. Pattern of distribution of assimilates in plants is markedly affected by the changes occurring during transition from vegetative to reproductive phase (Halevy and Mayak 1981) and the process controlling flower development regulates the partitioning of assimilates between the flower and rest of the shoot system. The developing flowers act as a sink and the rate of carbohydrate flow from source to sink is related to the rates of activities of enzymes like invertase. Developing corolla in roses continues to import dry matter throughout its development and reducing sugars and starch accounts for 50% of its dry weight (Ho and Nichols 1977). Therefore, hydrolysis of stored starch is probably associated with flower opening so far as a decrease in water potential favours influx of water with the corolla tissue and promotes cell enlargement. In our laboratory we have studied various physiological and biochemical aspects of these two species of rose during flower development. Such studies will be helpful to enlarge our insight of the flowering behaviour in finding out suitable indicators of various physiological processes which may help in enhancing essential oil production of both these scented roses.

Growth characteristics

Petal development can be divided into two main phases: first a slow growth phase resulting mainly from cell division and a second rapid growth phase resulting from cell expansion (Martin and Gerats 1993). Normally at the end of cell division the petals are only 35-40% in their final size and further growth and unfolding is primarily a result of differential cell elongation. Sood *et al*. (2006) studied extensively the various physiological and biochemical parameters in both the scented rose, viz. *R. damascena* and *R. bourboniana*. The fresh weight, dry weight and moisture content was maximum in both the rose species at the time of full bloom suggesting that cell expansion phase plays important role in the development of these flowers (**Fig. 4A-C**).

In most of the plants, anthocyanin synthesis in the flowers is under developmental regulation and its accumulation coincides with petal growth (Mol *et al*. 1996). Anthocyanin accumulation usually occurs at later stages of petal development and these stages are characterized by rapid growth resulting only from cell expansion (Ben Nissan and Weiss 1996). In both the rose species (Sood *et al*. 2006), anthocyanin was significantly high before flowers started to open and subsequently decreased at full bloom (**Fig. 4D**). Anthocyanin accumulation is an integral part of flower development in most plants and it seems to be regulated by same factors that control petal growth (Weiss 2000). In most plants, petal pigmentation takes place during the second phase of development and its accumulation is tightly linked to the process of cell expansion. However, there is no universal trend found in anthocyanin content in petals. The level stays stable in some flowers and declines drastically in

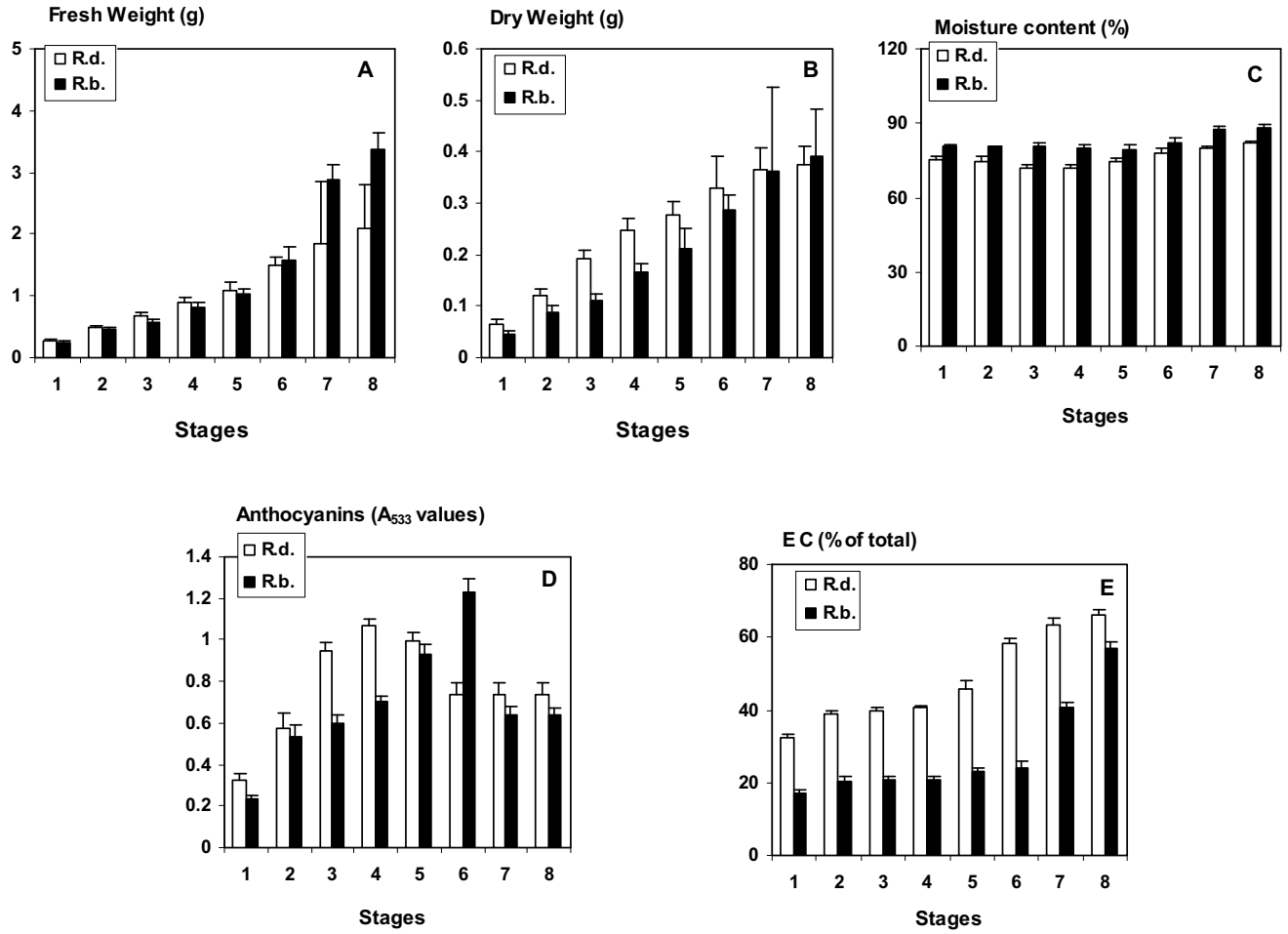


Fig. 4 Changes in (**A**) Fresh weight, (**B**) Dry weight, (**C**) Moisture content, (**D**) Anthocyanin, (**E**) Electric conductivity in petals of *Rosa damascena* (R.D.), and *Rosa bourboniana* (R.B.) during different stages of flower development. Vertical bars represent ± SEM (n= 3). (From **Sood S, Vyas D, Nagar PK** (2006) Physiological and biochemical studies during flower development in two rose species. *Scientia Horticulturae* **108**, 390-396, with kind permission from Elsevier).

others, while in some flowers a dramatic synthesis is evident (Sakata and Uemoto 1972; Sickland 1972). Increased electrolyte leakage from tissue is usually an expression of modifications in the physical properties of cell membranes (Shinitzsky 1984). The loss of membrane integrity measured by the leakage of ions increased continuously in the scented rose from the stage when the petal colour was intensified (**Fig. 4E**). Results by Sood *et al*. (2006) demonstrated that conductivity measurements of petal diffusates provide a sensitive indicator of physiological changes associated with the growth and development and flower opening of the two diverse species of rose. The changes in the rate of ion leakage from petals are considered to demonstrate changes in membrane permeability and membrane lipid/ protein composition (Serek *et al*. 1995).

Carbohydrates participate in a number of processes during the development of the flowers. The flower bud is a major sink for assimilates under favourable conditions, whereas a shortage of carbohydrates often leads to arrest of development. Since the petals are nearly achlorophyllus organs, very little photosynthesis occur in them and they depend entirely on adjacent tissues/leaves for carbon source and osmotic components (Harris and Jaffcoat 1972). Reducing sugars rather than sucrose were noted as the main constituents of sugar pool of the mature petals (Nichols 1973). Several studies have shown that sugar level increases during petal development to a maximum at the stage of rapid cell expansion (Clement *et al*. 1996). The content of reducing sugars in younger petals was lower and increased rapidly in both the species (Sood *et al*. 2006) from the stage when petals started to split reaching to maximum at full bloom (**Fig. 5A**). The role of sugars in flower development may be multifunctional: they act as an energy source (Moalem-Beno *et al*. 1997) osmotic regulators (Bieleski 1993) and as precursors for metabolic processes and the increased osmoticum in the petals have been suggested to be the driving force for their expansion. It has been suggested (Ho and Nichols 1977) that soluble sugars in rose flowers are important as osmotically active substances in promoting petal growth and this finding is substantiated by the findings of Sood *et al*. (2006) for both the species of rose. Many studies have shown that sugars act as signalling molecules in higher plants (Neta-Sharir *et al*. 2000) and in most cases sugar phosphorylation by hexokinase is required to initiate signal transduction (Jang and Sheen 1997; Penna *et al*. 2006). The promotive effect of sugars on petal growth seems to be a general phenomenon. However, it is still not clear whether sugars act in all cases as specific signaling molecules to promote gene expression or via another mechanism.

Starch in the petals acts as a reservoir of carbon at times of need. Sood *et al*. (2006) noticed a steady decline in starch content as the flower of both rose species reached their full blooming stage (**Fig. 5B**). Similar findings were observed during maturation of the perianth leaves of gladiolus flowers (Ferreira *et al*. 1986) and in ornamental roses (Ho and Nichols 1977). In carnation, the starch content in petals declined as the flowers developed (Tirosh and Mayak 1988); however, the extent of decline was greatest in cut flowers held in water, least in attached and intermediate in flowers held in sucrose solution. Further, as a function of stages of flower development in carnation, starch content decreased and soluble sugars increased concomitantly and hence, increased sink strength of the developing flower is coordinated with increased mobilization of stored reserves as well as supply of photosynthates. Sood *et al*. (2006) found

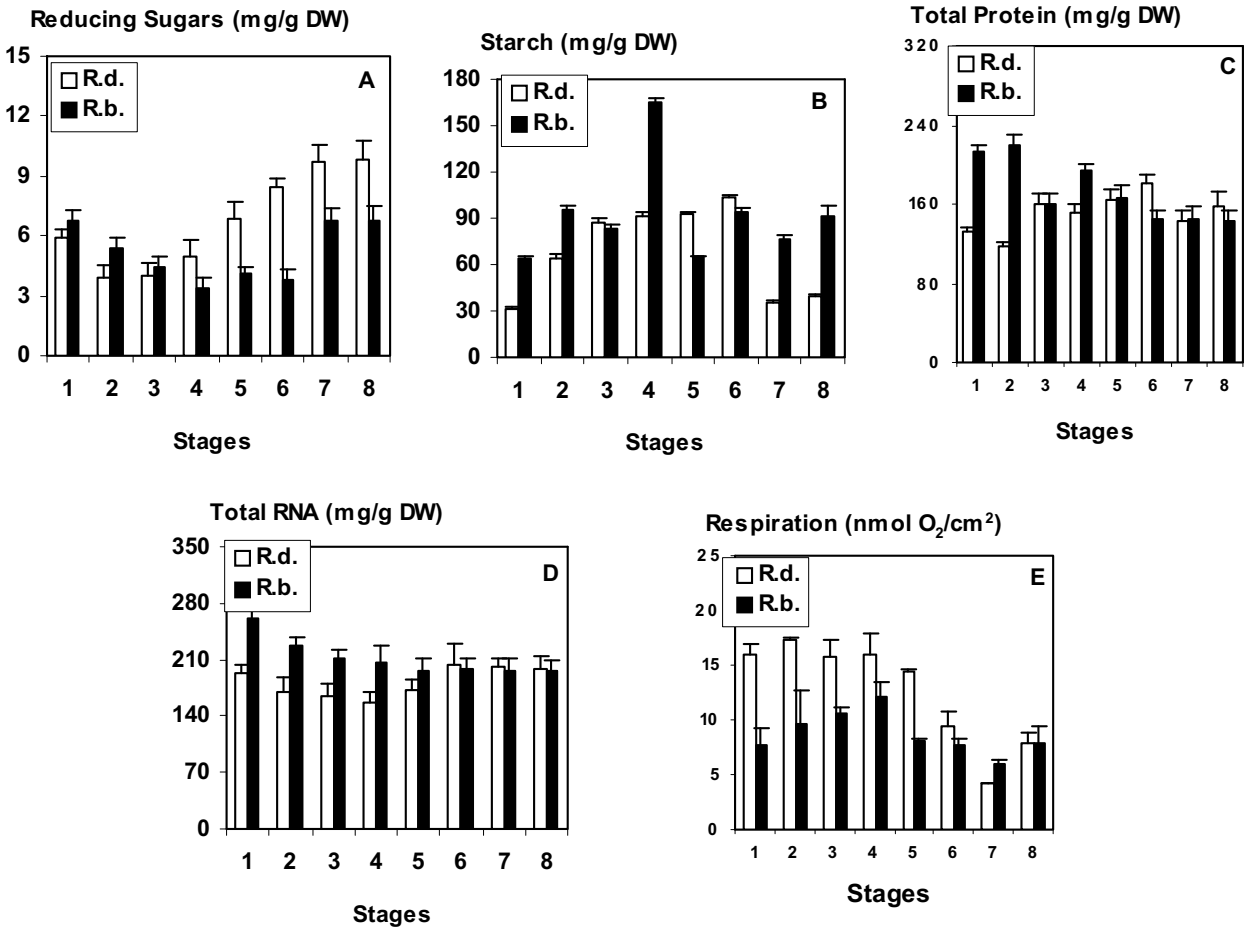


Fig. 5 Changes in (**A**) reducing sugars, (**B**) starch, (**C**) total protein, (**D**) total RNA, (**E**) respiration in petals of *Rosa damascena* (R.D.), and *Rosa bourboniana* (R.B.) during different stages of flower development. Vertical bars represent ± SEM (n= 3). (From **Sood S, Vyas D, Nagar PK** (2006) Physiological and biochemical studies during flower development in two rose species. *Scientia Horticulturae* **108**, 390-396, with kind permission from Elsevier).

that the amount of protein per unit dry weight and RNA was highest in the youngest stage and lowest in the later stages of development in both the species of rose (**Fig. 5C, 5D**). A loss of protein has been reported during development in daylily petals (Ley-Yee and Stead 1992) and *Sandersonia* flowers (Eason and Webster 1995). Loss of RNA can be due to decrease in the rates of synthesis or increase in the activity of RNase but the latter increase can be large. In *Ipomoea*, RNA contents start decreasing just before full bloom, whereas the sharp increase in RNase is evident only after beginning of colour fading indicating that the first stage in decline of RNA is caused by reduced synthesis (Matile and Winkenbady 1971). It has been shown that while the total protein content of the petals declined in morning glory (Banmgartner *et al*. 1975), some hydrolytic enzymes like RNase, protease, and β-glucosidase were synthesized *de novo* indicating the requirement of protein synthesis and the breakdown products of the cell's macromolecules which could be transported out of the petals to other parts of the plant (Wagner and Siegelman 1975). The rate of respiration in many flowers rises to a maximum during the early stages of growth and development followed by a gradual decline as the flowers start to open. Similarly, in both *R. damascena* and *R. bourboniana* high respiration rate was noticed during the early stages of petal growth (**Fig. 5E**) which gradually declined after the end of sepals completely separated from each other till half open stage; however, during full bloom it again increased in both the species (Sood *et al*. 2006). The gradual decline in respiration may be caused by a short supply of readily respirable substrates and the content of these may indicate the potential life of the flowers at a specific temperature (Nichols 1973). The size of the respirable substrate pool, which is composed mostly of sugars is affected by the rate of hydrolysis of starch and other polysaccharides (Ho and Nichols 1977) translocated to the petals on the one hand (Nichols and Ho 1975) and translocation out of the flower to other plant parts on the other. The gradual decline in respiration of rose petals and decrease in respiration efficiency may be due to progressive inability of mitochondria to utilize the substrate.

The role of peroxidase (POX) in any system is complicated by the postulated ability of the enzyme to produce lignin and to reduce growth by cross-linking wall materials (Lee and Lin 1995). High POX activity in developing apple flowers and fruits (Abbasi *et al*. 1988) was noticed during initial stages of flower development and decreased to 5-8–folds at full bloom while high activity in dormant buds was noticed which declined at time of bud break. The level of POX expression has been shown to be altered by several types of stresses such as wounding (Lagrimini 1991), environmental and chemical stresses and pathogen infection (Gasper *et al*. 1985) and also during low temperature acclimation. The increase in specific activity of POX in petals of both the rose species (**Fig. 6A**) during the early stages of development (Sood *et al*. 2006) may be due to two possibilities, i.e. induction by hydrogen peroxide and/or by the decrease in total protein. Bartoli *et al*. (1995) studied POX activity in carnation petals and found the similar results but the function of POX is still obscure. It was suggested that the enzyme functions in part to strength walls of the vascular cells, which remain functional late into senescence (Panavas and Rubinstein 1998). Sood *et al*. (2006) observed a decrease in catalase activity at full bloom in petals of both rose species (**Fig. 6B**). However, an increase in activity during the stages before full bloom suggests an alternative source of H_2O_2 formation possibly through polyamines (PAs), since H_2O_2 is generated during polyamine oxidation (Angelini and Frederico 1989) and polyamine biosynthesis

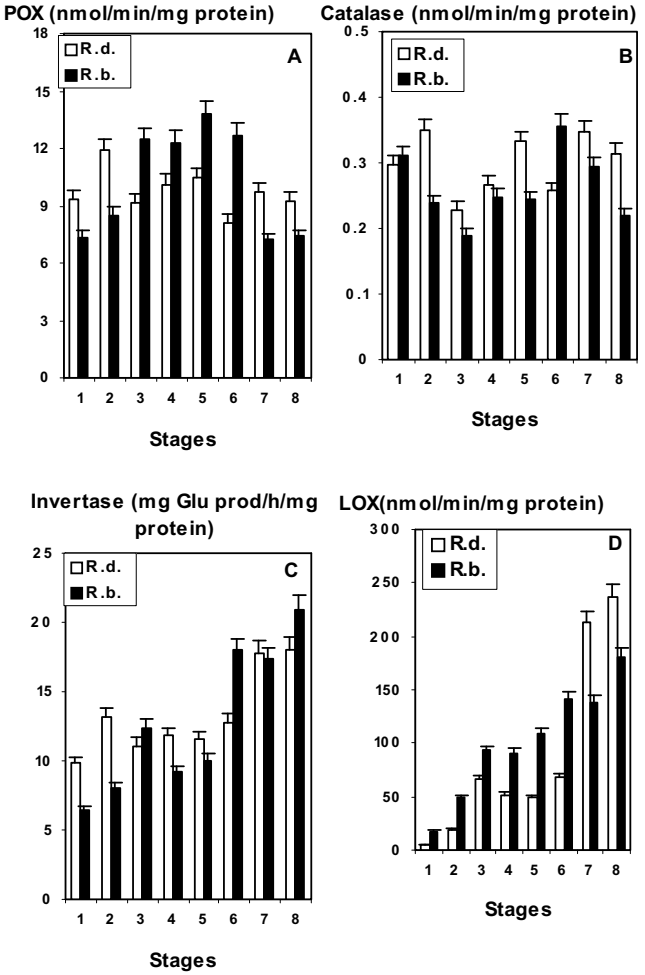


Fig. 6 Changes in (**A**) peroxidase, (**B**) catalase, (**C**) invertase, (**D**) lipoxygenase activity in petals of *Rosa damascena* (R.D.), *Rosa bourboniana* (R.B.) during different stages of flower development. Vertical bars represent ± SEM (n= 3). (From **Sood S, Vyas D, Nagar PK** (2006) Physiological and biochemical studies during flower development in two rose species. *Scientia Horticulturae* **108**, 390-396, with kind permission from Elsevier).

and oxidation are very active during flower development (Kushad *et al*. 1988). High catalase could perform a detoxifying function providing protection against cell damage caused by oxidative events (Patterson *et al*. 1984). Generally, flower petal tissue contains high activity of invertase and most of the sugar pool of mature petals is composed of inverted sugars (Wooden and Wang 1987). Large amounts of sugars are utilized as substrates for respiration, synthetic purposes and as an osmolyte for maintaining osmotic potential to expand petal cells. Ichimura *et al*. (1998) suggested that invertase probably plays an important role in determining the patterns of assimilate distribution in flowers. Invertase activity has also been correlated with dry weight gain in most of the floral organs of easter lily (Ranwala and Miller 1998). In palmarosa inflorescences (Dubey *et al*. 2003) soluble acid invertase enzyme was identified as the major sucrose catabolising enzyme playing an important role in providing carbon involving the process by which sucrose is being utilized for various biosynthetic purposes including essential oil biosynthesis. The high activity of invertase during the latter stages of flower development in the petals of both *R. damascena* and *R. bourboniana* (Sood *et al*. 2006) appears to be necessary in providing metabolites and energy to growing tissues for flower opening and various other biosynthetic (primary and secondary metabolic) processes including essential oil biosynthesis (**Fig. 6C**). Lipoxygenases (LOX) occur widely in higher plants and catalyse the deoxygenation of polyunsaturated fatty acids. The products of LOX catalysed reactions are further metabolized into molecules functioning in many biological processes (Siedow 1991). In a number of floral tissues such as *Rosa* hybrid (Fukuchi-Mizutani *et al*. 2000) LOX activity increases before the onset of electrolyte leakage (a marker of loss of semi-permeability). The product of LOX catalysed reactions hydroxy fatty acids and their free radicals are considered to cause membrane destruction and probably, these events may also occur in petals of both the rose species during the final stages of flower development (**Fig. 6D**, Sood *et al*. 2006). It is of interest to note that in both these species, immediately after full bloom the flower senesces very quickly. Further, the possible role of LOX may be in the biosynthesis of jasmonates, which are the end product of LOX pathway from α-linoleic acid (Sambdner and Parthier 1993) and methyl jasmonate has been reported to enhance senescence in *Petunia* and *Dendrobium* flowers (Porat *et al*. 1993).

Plant growth substances

Flower development involves a large number of interrelated growth, senescence and abscission processes and different plant hormones play distinct roles in the development of flower organs and their functions. The participation of a particular plant hormone in the regulation of development processes may be indicated by its correlative changes with the process being studied. Although not always studied in the same flowers, each one has been demonstrated to play a potential role in the hormonal controlling systems. It is generally accepted that this control is exerted through a balance between different hormones with each other and with other internal factors.

Abscisic acid

The plant hormone, abscisic acid (ABA) is considered to participate in the regulation of many aspects of plant growth and development and in response to various environmental stresses (Seo and Koshiba 2002) and many of these processes are correlated with endogenous ABA levels. The role attributed to ABA in flower is that of regulating the process of senescence. Mayak and Halevy (1972) studied ABA levels in rose petals cv. 'Golden-Wave' and found that the level of ABA increased as the flower aged indicating that it plays important role during senescence and that external application of ethylene accelerated the process and induced a rise in endogenous ABA-like activity. It was further suggested that the participation of these two plant hormones in the control of senescence is via the same pathway (Mayak *et al*. 1972). Muller *et al*. (1999) found differences in the endogenous ABA levels between the two miniatures rose cultivars. 'Vanilla' had a low ABA content at all flower developmental stages while ABA content of 'Broze' petals was high in buds, lower in open flowers, which increased during flower senescence. In certain other varieties of roses, ABA increases only during advanced stages of senescence (le Page-Degivry *et al*. 1991) and this is also observed in detached senescing leaves of tobacco (Even-Chen and Itai 1975) and rice (Philosoph-Hadas *et al*. 1993). Exogenous ABA inhibits the early phase of flowering in *Pharbitis nil* (Maeda *et al*. 2000) without influencing the time measuring process although endogenous ABA plays a very little role in regulation of flowering. In the petals of daylily, endogenous ABA increased before flowering and during flower senescence indicating that is an important component of senescence (Panavas *et al*. 1998). It has been suggested that differences in ABA content reflect tissue-specific variation in floral organs of *Citrus sinensis* L. (Harris and Dugger 1986). Sood and Nagar (2003a) studied changes in endogenous ABA in petals of *R. damascena* and *R. bourboniana* during flower development. A progressive increase in free ABA was observed during flower development till full bloom in both species with higher content of free ABA in *R. damascena* and a positive correlation was observed between endogenous ABA levels and flower development. The increase in the endogenous levels of ABA appear to provide support

for the suggestion that ABA is an important component of normal senescence of rose species. It is of interest to note that while bound ABA level increased in *R. damascena* till petals started to split, in *R. bourboniana* it continued to increase till full bloom. Although the physiological role of bound ABA is not clear, it is assumed to be an inactive storage form, utilized as when required by which free ABA is sequestered (Milborrow 1983). It has been postulated (Trewavas and Jones 1991) that there may be, as yet undetermined factors involved with the natural responses that are not recognized by ABA. In both the rose species the levels of acidic and bound phenols varied with flower development (Sood and Nagar 2003a) and that these could possibly play some role in the physiological changes of the plants. The phenolic compounds which were once thought to be mere secondary compounds, have been shown to aid in induction and development of flower buds. It has been suggested by the same authors that the endogenous ABA content in a given cell is determined as the sum of the rates of biosynthesis, catabolism and transportation. Hence, the components and molecular mechanisms involved in ABA perception and signal transduction, as well the regulation of gene expression by ABA, could be studied in two rose species in parallel with regulation of site determination of ABA synthesis and mobilization during flowering and senescence periods.

Polyamines

In last few decades, considerable attention has been given to the involvement of polyamines in different plant developmental processes (Kumar *et al*. 1997; Martin-Tanguy 2001; Kuznetsov and Shevyakova 2007). They have been shown to affect various physiological processes by modulating the transductions of pectic signals and are implicated in different plant growth and developmental processes like cell division, differentiation, embryogenesis, fruit set, fruit ripening and flowering (Galaston and Kaur-Sawhney 1995; Kakkar *et al*. 2000; Sood and Nagar 2004). In recent years, through the use of inhibitors of polyamine biosynthetic, and transgenic approaches, new insights into the role of polyamines in plant development processes have become apparent (Mamberg *et al*. 1998; Kakkar and Sawhney 2002). At cellular pH values they behave as cations and can interact with anionic macromolecules like DNA, RNA and phospholipids and certain proteins. In addition to commonly occurring polyamines, they can bind covalently to proteins and conjugates to hydroxycinnamic acids forming phenol amides (Martin-Tanguy 1997). These conjugated polyamines appear to function as storage pools to balance out the levels of free polyamines required for growth. It has been proposed that the increased accumulation of polyamine conjugates occurs in parallel with the enhanced activities of ornithine decarboxylase (Burtin *et al*. 1991), which is responsible for polyamine biosynthesis.

Polyamines also appear to be involved in the flowering process i.e. induction of flowering (Kaur-Sawhney *et al*. 1988) and the development of floral organs (Malmberg *et al*. 1985). The floral organ of the male sterile stamenless-2 mutant of tomato contain significant higher levels of polyamines than those of the wild type (Rastogy and Sawhney 1990). Changes in polyamine metabolism have also been correlated with floral development of tobacco (Martin-Tanguy 1997). The relationship between polyamine titres and flowering has also been validated using inhibitors of polyamine biosynthesis, e.g. DFMA, an inhibitor of ADC, inhibited flowering in tobacco TCL, while DFMO, an inhibitor of ODC, did not inhibit flowering (Tiburcio *et al*. 1988). The involvement of polyamines in floral induction has also been demonstrated by direct spray applications of compounds. Apple plants sprayed with different polyamines showed increased floral initiation and fruit set (Costa and Bagni 1983) while increased fruit set has also been reported in olives following application of putrescine (Put) at higher concentration during flowering (Rugini and Mencuccni 1985). On the other hand, polyamines inhibited flowering under inductive photoperiods in *Lemna* sp. (Tsao and Yin 1985), with spermine (Spm) exhibited the greatest effect and Put the smallest. In the flowers of *Citrus sinensis*, Put and spermidine (Spd) synthesis increased during the early developmental stages, followed by a decline and subsequent increase at anthesis (Kushad *et al*. 1990). However, in strawberry, free polyamines remained at a low concentration during flower development with phenylethylamine was the predominant amine, representing 80-90% of total free amine pool. In *Brassica rapa* a major net synthesis of free and conjugated polyamine particularly Spm occurred with flower opening and pollination (Puga-Hermida *et al*. 2003).

Changes in the concentrations of endogenous polyamines were determined by Sood and Nagar (2004) in both *R. damascena* and *R. bourboniana* during different developmental stages (**Figs. 7, 8**). High free Put and Spd concentrations were associated with early stages of flower development and then decreased in *R. damascena*. At full bloom, the concentrations of free Put was higher than rest of the polyamines measured. A higher ratio of Put to Spm was observed during flower development which supports the notion that cells are actively dividing, while a decline in the ratio and in total Put and Spm concentration indicates that tissue growth is primarily by cell enlargement (Bachach 1973). The concentrations of all the polyamines in the three fractions (free, conjugated and bound) were lower in petals

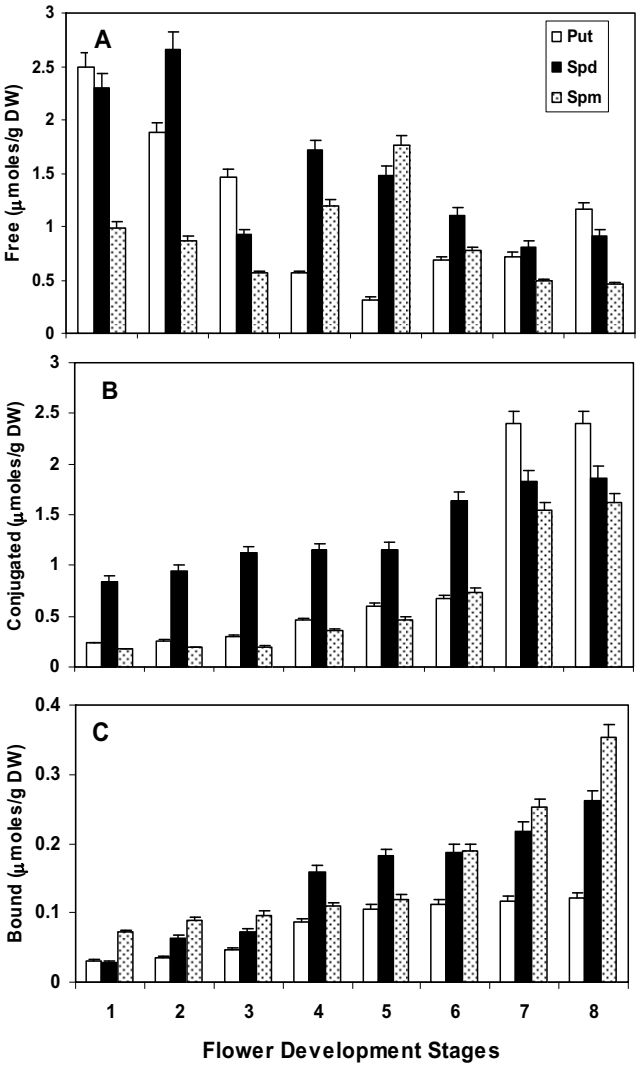


Fig. 7 Changes in the concentration of free (**A**), conjugated (**B**) and bound (**C**) polyamines in the petals of *Rosa damascena* during different stages of flower development. Vertical bars represent ± SEM (n= 3). (From **Sood S, Nagar PK** (2004) Changes in endogenous polyamines during flower development in two diverse rose species. *Plant Growth Regulation* **44**, 117-123, with kind permission of Springer Science and Business Media).

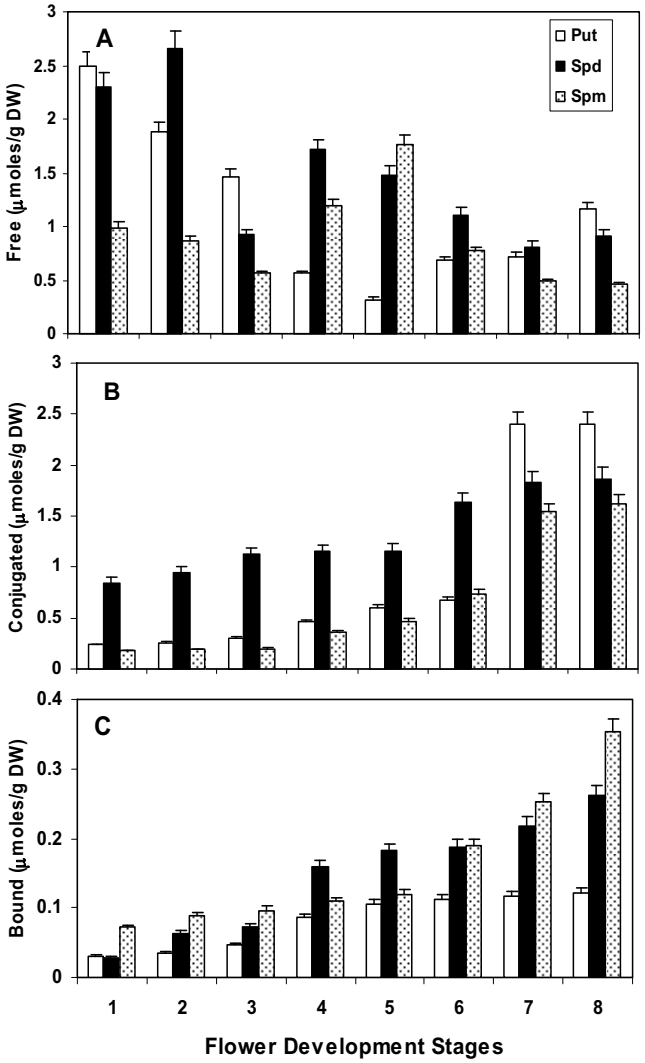


Fig. 8 Changes in the concentration of free (**A**), conjugated (**B**) and bound (**C**) polyamines in the petals of *Rosa bourboniana* during different stages of flower development. Vertical bars represent ± SEM (n=3). (From **Sood S, Nagar PK** (2004) Changes in endogenous polyamines during flower development in two diverse rose species. *Plant Growth Regulation* **44**, 117-123, with kind permission of Springer Science and Business Media).

Table 1 Putrescine titer (free) in leaf exudates of *R. damascena* and *R. bourboniana.* No conjugated putrescine was detected. Values are means of three replicates. Different letters on values show significant differences at $p < 0.05$.

	Putrescine n moles/ml root exudates		
Period	***Rosa damascena***	***Rosa bourboniana***	**Mean**
20 d before flowering	0.102 b	0.466 b	0.284 b
Peak flowering period	0.467 a	0.687 a	0.578 a
20 d after flowering	0.064 b	0.404 b	0.234 b
Mean	0.211 b	0.520 a	
CD (P<0.05)			
Species (S)	0.042		
Period (P)	0.051		
S × P	0.073		

From **Sood S, Nagar PK** (2005) Xylem and phloem derived polyamines during flowering in two diverse rose species. *Journal of Plant Growth Regulation* **24**, 36-40, with kind permission of Springer Science and Business Media).

Table 2 Putrescine titer (free) in root exudates of *R. damascena* and *R. bourboniana*. No conjugated putrescine was detected. Values are means of three replicates. Different letters on values show significant differences at $p < 0.05$.

	Putrescine n moles/ml root exudates		
Period	***Rosa damascena***	***Rosa bourboniana***	**Mean**
20 d before flowering	0.231 b	0.211 b	0.222 b
Peak flowering period	0.371 a	0.286 a	0.329 a
20 d after flowering	0.126 c	0.139 c	0.132 c
Mean	0.243 a	0.212 b	
CD (P<0.05)			
Species (S)	0.023		
Period (P)	0.029		
S × P	0.040		

From **Sood S, Nagar PK** (2005) Xylem and phloem derived polyamines during flowering in two diverse rose species. *Journal of Plant Growth Regulation* **24**, 36-40, with kind permission of Springer Science and Business Media).

of *R. bourboniana* (**Fig. 8**) than in *R. damascena* (**Fig. 7**) and genotypic differences between these two species may account for this. These authors also noticed a steady increase in all conjugated polyamines during entire period of flower development with predominance of Put at full bloom. In *R. damascena* the bound Spm was higher than rest of the polyamines during full bloom, while in *R. bourboniana*, similar situation was observed during the early stages of flower development, however, in the latter during full bloom the concentration of conjugated and bound Spm was higher than rest of the polyamines. It has been proposed (Martin-Tanguy *et al*. 1996) that polyamine conjugates have important functions in the floral induction, floral evocation and reproductive processes. Conjugation to cinnamic acids might be a way of regulating the free polyamine pools in plant cells and these conjugates may act as, means for polyamine translocation and that they could be the substrates for amine oxidases (Havelange *et al*. 1996). In a number of species during floral development, the conjugates are not metabolized and do not act as storage forms for polyamines and in these morphogenetic processes at least the major pathway of polyamine metabolism is via the conjugation. It has been proposed that the role of hydroxycinnamic amides and/or polyamines in flower physiology can be better understood using Ri T-DNA from *Agrobacterium* sp (Kakkar and Sawhney 2002). Although no apparent relationship can be made between specific hydroxycinnamic amide and bud formation, yet spermidine accumulation can be used as a physiological marker for floral induction.

As discussed above, the participation of polyamines during flowering has been strongly suggested by certain studies (Malmberg and Mcindoo 1983; Caffaro and Vicent 1995) and supporting evidence in the control of floral transition has come from the use of inhibitors of polyamine synthesis (Havelange *et al*. 1996). Polyamines are translocated in plant from roots to upper parts and *vice versa* leading to hypothesis (Caffaro *et al*. 1993) that long term translocation of these polycation may be both basipetal and acropetal. In young and adult plants of potato, basipetal transport of radioactivity was observed after feeding labeled PAs to cotyledons or mature leaves respectively (Beraud *et al*. 1992). This indicates that phloem sieve tubes are directly involved in the basipetal translocation of PAs. This translocation from leaves to axillary buds in soybean (Caffaro *et al*. 1994) has been considered part of the complex mechanism in flower signaling and the transition from vegetative buds to flowering buds. Polyamine contents in xylem (root) and phloem (leaf) exudates in both the scented rose species were analysed by Sood and Nagar (2005) before, during and after flowering during main flowering season i.e. April-May. Only free Put was detected in the xylem and phloem exudates at these time periods which was high during the peak flowering period. In phloem, Put content was significantly higher in *R. bourboniana* than *R. damascena* at all the three stages, whereas in the xylem exudates it was relatively higher in *R. damascena* at the peak flowering period (**Tables 1, 2**). Because only free PAs were translocated in the study, supports the concept that PAs conjugated to cin-

namic acids are sequestered in the vacuoles and therefore, are probably unable to enter the cytosolic fluid of the sieve tubes. The presence of PAs in the xylem implies that that they can be synthesized in the root system and exported to the shoot. Since in both the rose species Put content in the exudates varied considerably suggests that the physiological condition of the root system and of the shoot affects the formation and partitioning of PAs. The participation of Put in controlling flowering process has strongly suggested in some other studies (Caffaro and Vicente 1995). Higher Put content in both xylem and phloem during peak flowering period and its decrease thereafter indicates that in both the species the period during which Put is needed for flowering is short. Inhibition of flowering by spraying DFMO, an irreversible inhibitor of Put synthesis (**Fig. 9**), implies that Put may qualify as a component of flowering and because the inhibitory effect of DFMO was substantially reversed by an application of Put suggests that Put is necessary for flowering in both the species of *rose* (Sood and Nagar 2005). It is interesting to know that petal senescence in *R. damascena* is much faster than *R. bourboniana* and exogenous polyamines were more effective in retarding senescence of leaf discs of the latter than the former species (Sood and Nagar 2003b) which was reflected in changes in the reserve metabolites like protein, RNA, starch, total and reducing sugars. It was suggested by the authors that the effect of PAs on retarding senescence of leaves of both species of rose could be due to the interaction with RNA and inhibition of enzyme action or enzyme synthesis. Recently, Sood and Nagar (2008) studied changes in concentration of endogenous polyamine and ethylene during postharvest periods in both the scented species of rose. At full bloom, the concentration of free Put was significantly higher than rest of the polyamines and the concentration of all the polyamines decreased during subsequent periods up to 48h after full bloom in both the species. Similar situation was also observed in conjugated fraction. In both the rose species the concentration of ethylene showed higher levels during full bloom with maximum in *R. damascena*, which increased during postharvest periods. Ethylene production is closely associated with polyamine synthesis since both utilize the same precursor i.e. *S*-adenosyl-L-methionine (SAM) for their synthesis. Ethylene biosynthesis occurs by converting methionine to SAM by SAM synthatase. In polyamine synthesis, Put is synthesized from ornithine and arginine through their decarboxylase activities, which then used as a precursor, together with the aminopropyle group derived from SAM after decarboxylation to form Spm and spermidine (Evans and Malmberg 1989). In view of sharing a common precursor SAM the two biosynthetic pathway, it has been postulated (Pandey *et al*. 2000) that both ethylene and polyamine may regulate each other synthesis. This postulation is based on the fact that these two substances show opposite effects in relation to senescence and it is the balance between the two opposite roles, which is crucial for one or other developmental processes like flowering in the plant. The biochemical mechanisms, which have been suggested to explain the biosynthetic relationship between the two pathways, are (i) competitive demand for a limited pool of common precursor (SAM), and (ii) feedback inhibition of enzyme action system in one pathway by the product of the competitive pathway. However, the extent of linkage between PA and ethylene pathways remain a moot point and the significance of these mechanisms during the course of normal plant growth and development is still not clear. Moreover, the requirement of PAs and ethylene in primary plant metabolism is not in question; rather it is the degree of their relationship at both the physiological and biosynthetic levels that remains to be solved.

CONCLUSIONS

In view of the existing problems facing rose breeding, *in vitro* techniques and other physiological and biotechnological tools are likely to play a vital role in rose improvement

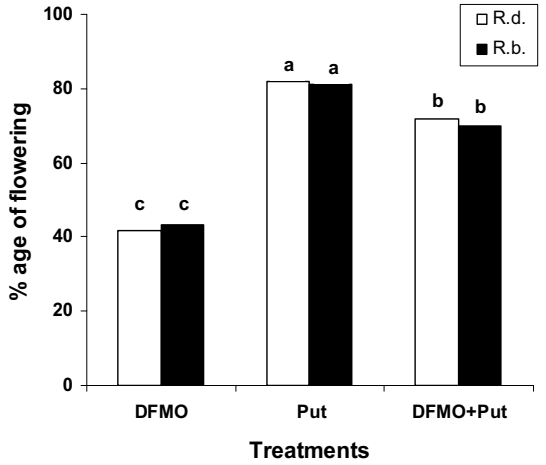


Fig. 9 Effect of DFMO (5 mM) and putrescine (5 mM) alone and when combined on the flowering of *Rosa damascena* (R.d.) and *Rosa bourboniana* (R.b.). Different letters upon the bar show significant differences at $p<0.05$. (From **Sood S, Nagar PK** (2005) Xylem and phloem derived polyamines during flowering in two diverse rose species. *Journal of Plant Growth Regulation* **24**, 36-40, with kind permission of Springer Science and Business Media).

programmes and introduction of new varieties. One such tool is *in vitro* propagation, which ensures rapid multiplication of elite clones and production of healthy, and disease free plants. *In vitro* haploidization will help in producing homozygosity and hence, overcoming the major problem of heterozygosity in cultivated roses. Embryo rescue will help in interspecific hybridization and thus production of fertile improved off springs. Further, the recent progress in genetic manipulation of plant cells has opened new possibilities for the improvement of rose. Protoplast fusion, leading to the production of somatic hybrid plants, is an approach in this direction for the introduction of novel traits into roses. The potential use of this technique for rose improvement includes the transfer of traits controlled by several genes. Further, it allows breeders to bypass sexual incompatibilities and hence facilitates widening of the gene pool available for rose improvement. In addition, both nuclear and cytoplasmic genomes from the different parental protoplasts are combined into a somatic hybrid plant. The resulting new nuclear/cytoplasmic combinations and the possibility of cytoplasmic gene recombination might lead to the modification of cytoplasmic traits. Further, conductivity measurements and membrane permeability changes provide a sensitivity indicator of physiological changes associated with growth and development and opening of flowers which is expected to provide leads in the flowering behaviour of two scented rose species which may be utilized to increase essential oil production.

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