Roles of plant growth regulators on flowering of rose (Rosa hybrida L.'Red Rose')

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Abstract. Rose is the most popular ornamental flower all over the world, which is used as garden plants and cut flowers. In the case of Rosa hybrida L. 'Red Rose', flowering provides the major developmental transition from the vegetative to the reproductive stage, and reproduction is one of the most important phases in an organism’s life cycle. In this study, the morphological and physiological changes during the flower development of rose, which is planted in the garden, and roles of plant growth regulators on the flowering of in vitro vegetative shoots of rose were analyzed. The development of a flower includes three stages: the shoot apical meristem, floral meristem, floral bud. Levels of cytokinin, auxins, and gibberellins increased in the transition of meristem from the shoot apical meristem to the floral meristem stage. Plant growth regulators have important effects on the shoot apical meristem cell division and flowering. The combination of 0.5 mg.L⁻¹ GA₃, 0.1 mg.L⁻¹ NAA, 2.5 or 3.0 mg.L⁻¹ BA to Murashige and Skoog (MS) medium induces the floral transition of the in vitro vegetative shoots with the highest percentage (41%) as well as growth and development in comparison to the other treatments after 10 weeks. Then, the in vitro floral meristem continuously developed into a flower bud after 12 weeks.

1. INTRODUCTION

Nowadays, the rose belongs to the Rosaceae family with about 200 species all over the world [1]. Roses with a huge variety of characteristics such as flower shape clour and fragrance are used as garden ornaments. They are also an enormous demand in the perfume and cosmetic industries. The rose has been grown for millions of years, is an important cut flower crop, and the plant adapted to diverse climatic conditions. The transition from vegetative stage to reproductive stage of growth is a significant critical event in the life of a plant. This transition depends on numerous factors, including both internal and external elements. In vitro flowering is influenced by plant growth regulators, such as auxins and cytokinins, has been early studied on flowering in tobacco in vitro by thin cell layers method [2]. In some other reports, the role of cytokinins on in vitro floral morphogenesis is also studied, as observed in rose (hybrid tea) cv. “First prize”, the highest percentage of flowering (45%) was obtained from the shoots culture on MS medium containing 3.0 mg/L BA, 0.1 mg.L⁻¹ NAA, and 30 mg.L⁻¹ sucrose after 12 weeks [3]. Another study reported that in vitro flowering induction of Rosa hybrida L. cv “Red Masterpiece” was observed on MS medium containing 2.0 mg/L BA after 9 weeks of culture [4]. Floral organogenesis in rose flowers of these plants has four concentric whorls of organs that are specified by the development from the outside to the center of the flower, in the sequence as follows: sepals, petals, stamens, and carpels followed by the genetic ABC model, that...
similar to *Arabidopsis thaliana* [5]. However, the effect of plant growth regulators on the *in vitro* flower development of rose are still obscure, in overcoming abnormal phenomena of flowering rose culture *in vitro*. Therefore, this study aims to understand the shoot development in *in vitro* conditions and rose quality, controlling important rose traits, and flower initiation development.

## 2. MATERIAL AND METHODS

### 2.1. Material
Floral shoots of rose (*Rosa hybrida* L. ‘Red Rose’) in the stage before fully blooming with dormant buds were collected from rose gardens in Dalat (Vietnam).

### 2.2. Observation of morphological changes
The developmental flowers were observed by eyes, that appear during stages. Morphological changes of shoot apical meristem during flower development were observed under a stereomicroscope and optical microscope (CKX41, Olympus, Japan) after the longitudinal cut through the shoot apical meristem and dyed with double staining carmine aluminum and iodine green.

### 2.3. Extraction, isolation, and quantitative analysis of endogenous phytohormones
The plant hormones, including auxin, cytokinin, gibberellin, and abscisic acid in shoots at different stages of flowering from the garden, were extracted by using methanol and diethyl ether. The plant hormones were isolated by using silica gel thin-layer chromatogram (60 F254, 105554, Merck), at 29 °C with solvent chloroform: methanol: acetic acid (80:15:5 in volume). The plant hormones were detected under ultraviolet light [6]. Plant hormone level was measured by bioassay technique: Lettuce hypocotyl (*Lactuca sativa* L.), cucumber cotyledons (*Cucumis sativus* L.), Oryza coleoptile sections (*Oryza sativa* L.) were used to evaluate activities of gibberellin, zeatin, auxin, and abscisic acid, respectively. The hormonal level in each sample was measured in three replicates [7].

### 2.4. Effect of plant growth regulators on *in vitro* flowering from vegetative shoots
All nodal sections in positions from 3 to 5 (from flower buds down) containing vegetative shoots were excised. The nodal sections were washed out under running water in 30 minutes, then sterilized with 0.1 % HgCl₂ for 15 minutes. Thereafter, the explants were rinsed 3 times with sterile distilled water. Then, damaged tissues were aseptically separated from the nodal sections. The shoots with 0.5 cm in height were placed on MS [8] medium supplemented with 0.5 mg.L⁻¹ BA, 0.1 mg.L⁻¹ NAA, 0.5 mg.L⁻¹ GA₃, and 30 g.L⁻¹ sucrose [9].

After 30 days, the shoots approximately 2.0 cm in height were obtained from the shoot cluster and cultured to MS medium supplemented with 0.1 mg.L⁻¹ NAA, 0.5 mg.L⁻¹ GA₃, BA at different concentrations (0.5; 1.0; 1.5; 2.0; or 3.0 mg.L⁻¹), and 30 g.L⁻¹ sucrose. A few rose traits such as plant structure, flower development observing in all treatments.

### 2.5. Experimental design and statistical analysis
All experiments were set up under the controlled condition of fluorescent light with an intensity of 27 μmol.m⁻².s⁻¹, temperature of 23 ± 2 °C, relative humidity (60 - 70 %), and photoperiod 12 h/day. The experiment was repeated 3 times and each treatment was replicated in ten culture vessels. The data were analyzed by using Statistical Package for the Social Sciences (SPSS), version 16.0 for Windows.

## 3. RESULTS AND DISCUSSION

### 3.1. Morphological changes of the rose flowering
After cutting rose branches, shoots develop and form a flower bud. There are three main stages in the development includes (Figure 1): (1) Vegetative shoot: the shoot apical meristem is formed about 14 days after cutting rose branches. At this stage, the shoot apical meristem (SAM) mainly produces leaves (Figure 1a, b). (2) Flowering initiation: day 14 through 21, the SAM becomes the inflorescence shoot apical meristem. At this stage, the flower primordium becomes meristem maintenance and determinacy (Figure 1c, d). (3) Floral bud: from day 21 to day 31, branch rose continues to lengthen relatively. The floral bud as well as a floral meristem that appears during this stage. The sepals grow to completely cover the floral bud, petals, and sepals continue expanding until SAM has disappeared (Figure 1e, f).

![Figure 1](image.png)

**Figure 1.** Morphological changes of apical meristem in flower development: (a) shoot after 14 days. (b) the SAM after 14 days. (c) floral bud after 21 days. (d) the FM with sepals, petals after 21 days. (e) the floral bud after 47 days. (f) the FM with flower organs after 31 days (in this figure se: sepals; p: petals; and c: carpel).

### 3.2. The changing of plant growth regulators in shoots during flower development of the flowers from the garden.

In the transition of shoots from the vegetative growth to the flowering initiation stage, the level of auxins, cytokinins, and gibberellins of the shoot were increased. In the floral bud stage, the level of auxin continues increasing, cytokinin and gibberellin activity maintains the same level at this stage. The ratio of cytokinin/auxin in each stage was decreased. Meanwhile, the level of abscisic acid did not change (Table 1).

| Development stages          | Level of plant growth regulators (mg.L⁻¹) |
|----------------------------|-------------------------------------------|
|                            | Auxin | Cytokinin | Gibberellin | Abscisic acid | Cytokinin/auxin |
| Vegetative shoot           | 0.58  | 1.47      | 2.54        | 3.83          | 2.53           |
| Flowering initiation       | 1.12  | 1.73      | 3.64        | 3.85          | 1.54           |
3.3. Influence of plant growth regulators on in vitro flowering from vegetative shoot cultures.

After 10 weeks of culture, the results showed that floral bud was the highest proportion significantly when using BA 2.5 mg.L\(^{-1}\) and 3.0 mg.L\(^{-1}\), which higher compared to 1.5 mg.L\(^{-1}\) and 2.0 mg.L\(^{-1}\) (25.27%; 35.37 %, respectively). BA 0.5 mg.L\(^{-1}\) in this experiment did not improve flowering efficiency, which is no floral bud (Figure 2). In terms of the height of shoot, the treatment with the ratio of BA/NAA 5 and 10 was the highest number (Table 2). The BA/NAA ratio was an important factor for in vitro flowering and BA was the key in the transition of shoots from the vegetative to the reproductive stage of buds.

| Plant growth regulators (mg.L\(^{-1}\)) | Ratio of BA/NAA | Height of shoot (cm) | Flowering (%) |
|----------------------------------------|-----------------|---------------------|---------------|
| GA\(_3\) NAA BA                        |                 |                     |               |
| 0.5 0.1 0.5                            | 5 10 15 20 25   | 8.90 ± 0.21\(^a\)  | 6.77 ± 0.27\(^d\) |
|                                        |                 | 8.27 ± 0.15\(^{ab}\) | 25.27 ± 2.98\(^c\) |
|                                        |                 | 8.00 ± 0.29\(^b\)  | 35.37 ± 1.18\(^d\) |
|                                        |                 | 7.27 ± 0.15\(^c\)  | 39.91 ± 0.83\(^a\) |
|                                        |                 | 6.03 ± 0.29\(^d\)  | 40.97 ± 0.62\(^e\) |

Values with different letters in the same row are significantly different according to Duncan’s test (p=0.05).

3.4. Floral organization from vegetative shoots on MS medium containing GA\(_3\) 0.5 mg.L\(^{-1}\), NAA 0.1 mg.L\(^{-1}\), and the different combinations of BA.

| Plant growth regulators (mg.L\(^{-1}\)) | Ratio of BA/NAA | The presence of floral organs at a different time (weeks) |
|----------------------------------------|-----------------|----------------------------------------------------------|
| GA\(_3\) NAA BA                        |                 | 6 8 10                                                    |
| 0.5 0.1 0.5                            | 5 10 15 20 25   | Vegetative Vegetative Vegetative Sepal, petal Sepal, petal Stamen, carpel |
|                                        |                 | Stamen, carpel Stamen, carpel Stamen, carpel              |

Differentiated shoots were obtained after 10 weeks at all the treatments. On MS medium containing The BA and NAA with the ratio of 25 or 30, the flower primordia established the sepals and petal primordia were at 6 weeks of culture, which is in setting up organ identity early in flower development, compared to other treatments in the experiment. After 8 weeks of culture, flower morphogenesis of floral organs with sepal, petal, stamen, and carpel (Table 3). After 10 weeks, flowers comprise four different organs of concentric circles. In addition, lower ratios of BA and NAA (10, 15, and 20) had delayed flower organogenesis, compared with high rates of BA and NAA (25 or 30) after 8 weeks (Figure 4 b, c, d). In vitro flowering has petal numbers, and red color, which was less than ordinary flowers in the garden (Figure 3, 4).
Figure 2. Floral formation of bud development after 10 weeks of culture on MS medium supplemented with 0.1 mg.L\(^{-1}\) NAA, 0.5 mg.L\(^{-1}\) GA\(_3\) and BA at different concentrations: (a) 0.5 mg.L\(^{-1}\) BA, (b) 1.0 mg.L\(^{-1}\) BA, (c) 1.5 mg.L\(^{-1}\) BA, (d) 2.0 mg.L\(^{-1}\) BA (the red arrow indicating floral bud).

Figure 3. Floral morphogenesis in vitro flower with four concentric whorls of organ development after 12 weeks of culture on MS medium supplemented with 0.1 mg.L\(^{-1}\) NAA, 0.5 mg.L\(^{-1}\) GA\(_3\) and BA at different concentrations: (a) 1.0 mg.L\(^{-1}\) BA, (b) 1.5 mg.L\(^{-1}\) BA, (c) 2.0 mg.L\(^{-1}\) BA.
3.4. Discussions

In the floral transition, the shoot apical meristem (SAM) is converted into an inflorescence meristem (IM), which subsequently generates a floral meristem (FM), terminates after producing a defined number of floral organs [10]. In the transition of shoots from the vegetative to flower primordium stage, the level of endogenous cytokinin and gibberellin increased while cytokinin/auxin ratio was decreased, which in turn leads to the formation of flowers with an increased number of petals.

The role of cytokinin in morphogenesis is promoting the transition from undifferentiated stem cells to differentiation and expansion, especially floral morphogenesis. According to Corbesier et al. (2003) applied cytokinins induce molecular changes associated with the floral transition [11,12]. Auxin is crucial to plant morphogenesis establishment and various physiological processes, like the organ differentiates, cell expansion, floral opening, organ abscission. Moreover, the function of auxin during floral opening is to trigger flower formation in the shoot apex [13]. The plant hormones auxin and cytokinin, which control the timing of sepal initiation, organ initiation, together with flower organ development and maturation, contribute to increased flower size [14]. Other studies also have shown the cytokininins and auxins effects on in vitro flowering such as the percentage of vegetative to the
reproductive transition of flowers [15]. Therefore, a low cytokinin/auxin ratio and high levels of GA, which trigger floral meristem formation.

According to the ABC model of flowering, the A genes are necessary for sepal specification. A and B genes are necessary for petal specification, while stamen specification depends on the activities of B and C genes, and finally, carpels are specified by the C [16]. When plant growth regulators were supplemented to the in vitro culture medium, affecting the expression of the floral meristem identity genes and the genetic control of the flowering time. It’s indirectly stimulated the expression of genes forming floral organs followed by the ABC model [17]. In Rosa sp., the transition of shoots from the SAM to FM required a high cytokinin/auxin ratio for floral bud induction. The floral initiation stage of organs requiring higher concentrations of BA resulted in a higher flower bud induction (40.97 %). Noticeably, many abnormal flowers were observed, abnormal organs, including smaller or absent flowers, the color of flowers was decreased on different medium with the decrease concentration of cytokinins and a change of number in the floral organ by transducing signals from plant growth regulators, such as auxin and cytokinin. Several mutations that affect the number, size, and shape of one or several floral organs have also been characterized. The flowers from the high concentration of BA treatments are large blooming, and having beautiful colors. From that, it can be seen that BA in in vitro culture medium help improve the quality of rose plantlets cultured in vitro.

4. CONCLUSION
The results of this study showed the FM, which develops from SAM upon completion of the floral transition, terminates after producing a defined number of floral organs. In vitro flowering bud formation of rose, the primordial rise of the floral organs required a high cytokinin/auxin ratio for the early showing formation of sepals, petals, stamens, and gynoecium. The highest percentage (41%) of flowering was obtained on MS medium supplemented with 3.0 mg.L⁻¹ BA, 0.1 mg.L⁻¹ NAA and 30 g.L⁻¹ sucrose.

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