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Research Article

Effects of 5-ALA and Aqueous Organic Solvents Foliar Spraying on the Quality of Rosa Rugosa

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Abstract: Foliar spraying is employed in this study to test the effect of 5-aminolevulinic acid (hereafter 5-ALA), aqueous-organic solvents and their compounds on the quality of Rosa rugosa. The results showed that, compared with the control group (foliar spraying of fresh water), foliar application of 5-ALA or aqueous-organic solvents singly, or in combination, all have various improving effect on rose leaf area, flower diameter (indicating size of the flower), stem length, opening rate, flower color and the total biomass; foliar spraying of 5-ALA and aqueous-organic solvents jointly or mere 5-ALA both can increase the chlorophyll content of unit area leaf, while mere aqueous-organic solvents will decrease the unit area leaf chlorophyll content. The conclusion is that, joint foliar spraying of 5-ALA and aqueous-organic solvents will lead to the optimal result on the quality of Rosa rugosa by way of making full use of the mutually beneficial interaction between 5-ALA and aqueous-organic solvents. This finding no doubt, will shed light on the improvement of Rosa rugosa quality and benefit the future development of the Rosa rugosa industry.

Keywords: 5-aminolevulinic acid, aqueous-organic solvents, foliar spraying, rosa rugosa

INTRODUCTION

Rosa rugosa thunb, ranking among the most famous “top four cut-flowers”, plays an essential role in the world flower industry (Chen *et al*., 2010; Park *et al*., 2011). China, as the No.1 supplier of *Rosa rugosa* in the world, has gained considerable economic interests from Rosa rugosa thunb production. Product quality, for the moment, has become the urgent concern with the large scale of supply. In the year of 2011, the first-class standards for Rosa rugosa was issued by the Ministry of Agriculture of the People’s Republic of China: the fresh flowers shall be in its full blossom with neat peripheral petals and no withering brims and color fade; the well chosen erect stems are uniform and elegant, with the flower diameter being within the range of 30～45 cm varying with the different breeds; the stems shall be robust and straight, weighting above 40～50 g and the glossy leaves shall be even, green and shining. As a consequence, great attention shall be paid to the quality of Rosa rugosa in terms of the improvement of blossom, leaf, stem length and opening rate and so on.

Foliar spraying, as a regular measure in the fields of agriculture, forestry and flower industry, can significantly contribute to the growth of plants and improve their quality (Abdelgadir *et al*., 2009; Eidyan *et al*., 2014; Mohammadi *et al*., 2014). 5-aminolevulinic acid (hereafter 5-ALA) is considered as one type of plant growth regulator, the common precursor of chlorophyll, heme, vitamin B12 and some other tetrapyrrole compound in the organism (Hotta *et al*., 1997) and it can take part in the biosynthesis of chlorophyll and the adjustment of plant growth (Hotta *et al*., 1998; Ali *et al*., 2013). In agriculture, forestry and flower industry, high-concentration 5-ALA is a kind of biodegradable herbicide, pesticide and plant growth regulator (Kumar *et al*., 1999), while 5-ALA of low concentration can both contribute to plant growth and improve their quality (Sasaka *et al*., 1995) and improve their salt resistance and cold tolerance (Youssef and Awad, 2008; Hotta *et al*., 1998; Watanabe *et al*., 2000). Currently, 5-ALA has been widely applied in the world, while studies about its effect on the quality of followers when combined with aqueous-organic solvents have not yet found. This study, by way of foliar spraying experiments, explored the impacts of 5-ALA, aqueous-organic solvents and their compounds on the quality of Rosa rugosa, shedding light on the improvement of Rosa rugosa, so as to push the development of Rosa rugosa production industry.

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Table 1: Chemical property of field soil

Index	TN (g/kg)	AN (mg/kg)	SOM (g/kg)	AP(mg/kg)	AK (mg/kg)	EC (ds/m)	pH
Content	0.43	67.94	14.22	18.53	93.87	0.32	7.8

TN: Total Nitrogen; AN: Available Nitrogen, SOM: Soil Organic Matter; AP: Available Phosphorus; AK: Available Potassium; EC: Electrical Conductivity

MATERIALS AND METHODS

Study site: The experiment is carried out in the rose seedbeds at the demonstrative breeding base of new breed of lily through bulb reproduction in Shunyi District, Beijing and the soil at the base is derived from river alluvium with sandy loam texture. Two meters below the earth, there is no clay layer and the soil nutrients conditions are favorable. Table 1 shows data of chemical property of soil.

Materials: Rosa rugosa with the average length of 20 cm are chosen as the material.

Aqueous-organic solvents: OM $\geq$ 20 g/L; $N+P_2O_5+K_2O \geq 240$ g/L; Mg$\geq$11 g/L; Fe+Mn+Zn+B$\geq$2 g/L.

5-ALA solution: 5-ALA $\geq$ 0.3 g/L.

5-ALA, aqueous-organic solvents compounds: OM $\geq$ 20 g/L; $N+P_2O_5+K_2O \geq 240$ g/L; Mg$\geq$11 g/L; Fe+Mn+Zn+B$\geq$2 g/L; 5-ALA $\geq$ 0.3 g/L.

Methods:

Experiment design: A complete random block design is followed in the experiment. 4 divisions are set up and the roses are planted by cutting on the ridges one month before the experiment with the density of 15 cm×20 cm. The ridge is 0.5 m wide and intervals between the ridges are 0.5 m. Each division area is 3 m×8 m and 8 ridges are set up in it. Each division is repeated 3 times with 1 m's break between the adjacent two divisions:

- **T1:** Foliar spraying of the 800 times diluted compound fertilizer of 5-ALA and aqueous-organic solvents.
- **T2:** Foliar spraying of the 800 times diluted 5-ALA solution with 0.3 g/L.
- **T3:** Foliar spraying of the 800 times diluted aqueous-organic solvents.
- **CK:** Foliar spraying of the equivalent amount of fresh water.

High-concentration fertilizer will do harm to the rose leaf. Given the effective scope and the harmful concentration range of some microelements are very narrow, the concentration of the treatment solution shall be strictly controlled. Foliar application of the 800 times diluted treatment solution will promise no harm to the rose leaves and at the same time the nutrients within it will be efficiently absorbed. The application of each treatment solution is 3000 L/hm. The foliar spraying starts two months before the flowering and one more time every two weeks and it lasts 4 times in total. During the experiment, routine measures such as insect pest prevention and watering and so on are carried out as usual.

Calculation of the flowers: Six days after the fourth foliar spraying, the flower number of each rose is calculated. According to the branch number, roses are categorized into single branch trees, double branch trees and triple branch trees. 4 rows in the middle of each division are chosen as samples. In each sample area, after the flower number and rose tree number are calculated respectively, the following equation is employed to work out the respective flower number of each type of rose trees: "flower number = total flowers/total trees" and then the weighted mean value of the flower number of each tree of the three categories in each treatment is worked out and that is the average flower number of each rose tree.

Leaf area, flower diameter, stem length and their biomass measurement: Six days after the fourth foliar spraying, calculation of the leaf area, flower diameter (indicating the flower size), stem length and their biomass are carried out. In each treatment, 20 trees of the three branch categories are randomly sampled from the 4 middle rows in each repeated division of each treatment. With the branches, flowers and leaves being clipped, the flower diameter, stem length and leaf area of the three categories are measured respectively. At the same time, their flower weight, fresh stem weight and fresh stem weight are measured. Finally, the weighted mean values of each item of the three categories are worked out as the final result. One issue that shall be taken into consideration is that, when the leaves are clipped, 2-4 mature leaves at the 1/3 upper part of the sunny side on each rose tree are chosen; when calculating the leaf area, make sure that leaves are put on the squared paper and their outlines are to be drawn on the paper in pencil and then each leaf area is calculated by way of counting the squares it takes.

Measurement of the chlorophyll content: Six days after the third foliar spraying, chlorophyll contents are extracted with the mixture of 80% Acetone, 95% ethanol and distilled water and measured by model 751 spectropolarimeter (Wang, 2006).

Measurement of anthocyanin: It is on the sixth day after the fourth foliar application that anthocyanin contents are measured. Anthocyanin in the fresh flower is extracted with 0.1% HCL-methanol synthesis and its relative content is measured with model 751 spectropolarimeter (Agrochemistry Professional of SSSC (Soil Science Society of China, Agricultural Chemical Committee), 1983).

Statistical analysis: In this research SPPS17.0 is employed to conduct the data analysis, while Microsoft Excel is taken for tables, figures and charts drawing, PLSD for the significance of difference.

RESULTS

Effect on biomass: As shown in Table 2, soil fertility in the study site contributes 85.77% of the total increase of the rose biomass, while that of the fertilizer's is 14.23%, which indicates that foliar spraying has an obvious effect on the productivity of the rose biomass. The total biomass in each treatment forms a significant contrast to the CK and the difference is $p<0.01$. The variation trend among each treatment is treatment 1>treatment 3> treatment 2>CK and the biomass increase in the three treatments are 16.6, 6.9 and 8.8%, respectively higher than that of CK (Table 2). It was found that the increase rate in T1 (16.6%) is even higher than the sum of T1 and T2 (15.7%). The results showed that 5-ALA and aqueous-organic solvents could lead to mutually beneficial interaction when combined with each other and the contribution made by such an interaction to the total biomass is 0.9%.

Effect of different fertilizers on the fresh leaf area, flower diameter and stem length: All the three treatments could significantly ($p<0.05$) enlarge the rose leaf area, increase the flower diameter and stem length. To be specific, T1 is higher than T2 and T3 by 30.9% and 11.7% respectively; T3 is higher than T2 by 17.2%. In terms of effect on the flower diameter, T1 is higher than T2 and T3 by 15.9 and 8.1%, respectively, while T3 is higher than T2 by 7.2%. With respect to the effect on stem length, T1 is higher than T2 and T3 by 12.2 and 5.9%, respectively and T3 is 6.0% higher than T2 (Table 3).

Table 3 shows that The general variation trend of the effects on the leaf area, flower diameter and stem length from each treatment goes like this: T1>T3>T 2>CK, which shows that organic matter and various mineral nutrients are mainly responsible for the effect on the rose leaf area, flower diameter and stem length. When combined in foliar spraying, 5-ALA and aqueous-organic solvents promise an improvement on the unit area leaf, stem length and flower diameter. While applied separately, the sum of their increase rate on leaf area, flower diameter and stem length are 27.65, 23.4 and 15.2%, respectively while combined, the corresponding increase rates are 37.24, 25.0 and 17.3%, respectively. The results showed that the respective increase rates on rose leaf area, flower diameter and stem length, brought about by the mutual beneficial interaction of 5-ALA aqueous-organic solvents, are 9.59, 1.6 and 2.1%, respectively.

Effect on the flower number: Table 4 shows that the effect of different fertilizers on the flower number of the three rose categories follows the following general trend: T1>T3>T2>CK. There is no significant difference between T1 and T3 and the difference between T2 and CK is also insignificant. It reveals that, organic matter and various mineral nutrients are the leading factors responsible for the blossom of roses. In the three treatments, the flower number is higher than that of CK by 44.89, 12.24 and 29.25%, respectively, among which T1 is higher than T2 and T3 by 32.7% and 15.7% and T3 is 15.2% higher than T2 (Table 4). It can be found that the flower number of roses sprayed

Table 2: Effect of different fertilizers on biomass

Treatments	Fresh stem weight/g	Fresh leaf weight/g	Flower weight/g	Total biomass/g	Rate/%
T1	40.63±0.62 A a	0.39±0.04 A a	21.94±0.96 A a	62.95±1.44 A a	116.6
T2	37.60±0.79 BC bc	0.31±0.01 AB bc	19.82±0.28 A b	57.72±0.60 B b	106.9
T3	38.33±0.67 B b	0.36±0.05 AB ab	20.04±1.20 A b	58.77±1.05 B b	108.8
CK	36.59±0.65 C c	0.30±0.01 B c	17.11±0.67 B c	53.99±1.22 C c	100

$F_{0.05}$ = 4.07, $F_{0.01}$ = 7.59. The data in Table 2 were mean±SD, with the different capital letters and small letters in the same volume indicating significant difference at α = 0.01 and 0.05 level respectively, the same below; T1: Foliar spraying of the 800 times diluted compound fertilizer of the 5-ALA and aqueous-organic solvents; T2: Foliar spraying of the 800 times diluted 5-ALA solution with 0.3 g/L; T3: Foliar spraying of the 800 times diluted aqueous-organic solvents; CK: Foliar spraying of the equivalent amount of fresh water

Table 3: Effect of different fertilizers on fresh leaf area, flower diameter and stems length

Treatments	Leaf area/cm^2	Rate/%	Flower diameter/cm	Rate/%	Stem length/cm	Rate/%
T1	17.32±0.90 A a	137.2	8.0±0.1 A a	125.0	33.9±0.8 A a	117.3
T2	13.23±0.41 C c	104.8	6.9±0.3 BC c	107.8	30.2±0.6 C c	104.5
T3	15.50±0.40 B b	122.8	7.4±0.8 B b	115.6	32.0±0.3 B b	110.7
CK	12.62±0.54 C d	100	6.5±0.5 C d	100	28.9±0.2 C d	100

Table 4: Effect of different fertilizers on the flower number

Treatments	Flower number of single branch	Flower number of double branches	Flower number of three branches	Average flower number	Rate/%
T1	1.48±0.14 A a	2.25±0.04 A a	2.67±0.29 A a	2.13±0.07 A a	144.9
T2	1.10±0.09 AB bc	1.79±0.25 AB bc	2.06±0.25 AB bc	1.65±0.14 BC b	112.2
T3	1.33±0.25 A ab	2.02±0.28 AB ab	2.37±0.30 AB ab	1.90±0.22 AB a	129.2
CK	0.99±0.09 B c	1.64±0.24 B c	1.79±0.21 B c	1.47±0.11 C b	100

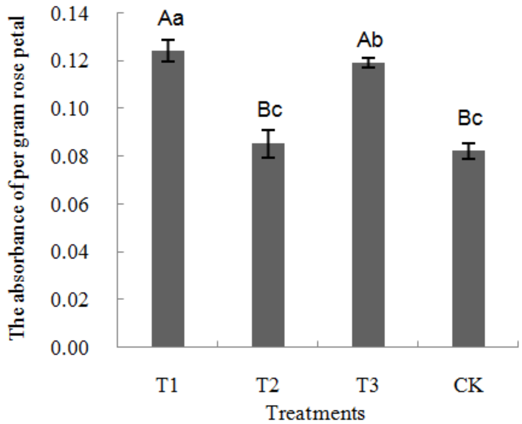

Fig. 1: Effects of different fertilizers on the absorbance of per gram rose petal

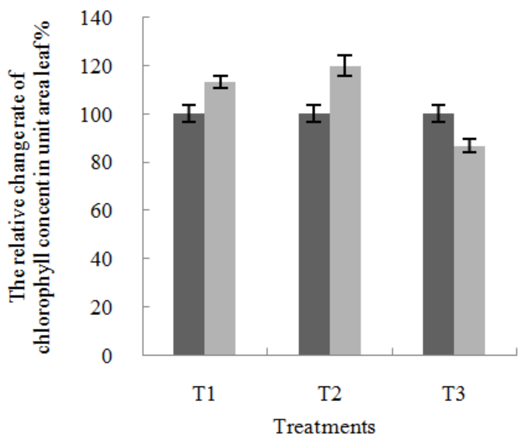

Fig. 2: The relative change rate of chlorophyll content in unit area leaf of different treatments

by 5-ALA and aqueous-organic solvents compounds ranks top and their increase rate (44.89%) is even higher than the sum of the two (41.49%) caused by their application separately. It indicated that 5-ALA and aqueous-organic solvents, when combined, can bring about reciprocal interaction in terms of fertility, which will increase the average flower production of each rose tree by 3.4%.

Effect on anthocyanin: the anthocyanin content in the rose petal varies with the absorbance of rose petal. The higher the absorbance, the more the anthocyanin content is and the darker the flowers are (Fig. 1). The results showed that, T1 and T3 could significantly increase the absorbance of per gram rose petal ($p<0.01$) and the difference between the two is significant ($p<0.05$). They are higher than the CK by 51.22% and 45.12%, while the difference in the absorbance of per gram rose petal between T2 and CK is insignificant. All of the data come to conclusion that the organic matter and the mineral nutrients are critical factors responsible for the improvement of rose flower color and in normal conditions, 5-ALA and aqueous-organic solvents in combination, are particularly beneficial to the improvement of flower color.

Effect on the chlorophyll content: All the three treatments have an impact on the chlorophyll content in unit area leaf and the overall picture goes like this: T2>T1>CK>T3. The unit area leaf chlorophyll content in T1 (0.017 mg/cm^2) and T2 (0.018 mg/cm^2) is higher than that of CK(0.015 mg/cm^2) by 13.33 and 20.00% respectively, while that of T3 (0.013 mg/cm^2) is 15.38% lower than that of CK. (Fig. 2). The result indicated that while 5-ALA can improve the unit area leaf chlorophyll content of roses, aqueous-organic solvents will lower it and that in terms of increasing the unit area leaf chlorophyll content, 5-ALA, while used singly, is more effective than combined with aqueous-organic solvents.

The analysis above indicated that the flourishing flowers of the Rosa rugosa, with the spraying of the 5-ALA and aqueous-organic solvents, are bigger, deeper in color and the stems are lengthy and robust, the big and thick leaves are rich with chlorophyll content. It also found that the mutual interaction of the compound of the two could profitably improve the effect of the fertilizers.

DISCUSSION

The comparison with soil fertilization showed that foliar spraying, as an efficient and effective way of fertilization, could provide nutrients directly through leaves and prevent the shortage of soil fertilization by way of protecting the microelements (such as Zn, Cu, Fe, Mn and so on) from being fixed in the soil, so as to guarantee the quality and productivity of the crops. The biomass, unit area leaf, flower diameter, stem length, flower color and opening rate improved significantly. The findings showed that, foliar spraying of organic matter and aqueous-organic solvents (such as macronutrient, grand and micronutrients) can contribute to the growth and the flower quality of Rosa rugosa, because organic matters will provide nutrients and active substance necessary for the plant growth, hence their quality and stress resistance. Among the essential mineral nutrients, N, P and K are the “macronutrients”, the primary ones affecting plants growth and their productivity and play an essential part in the improvement of plant quality. K is also known as “the quality nutrient” (Lu and Zhang, 2003). Among others, Fe is the component of some enzymes needed for the plants and Mn serves to regulate the oxidation-reduction equilibrium, contribute to the metabolism of N and do good to the plant growth. Zn, conducive to the formation of auxins and enhancement of photosynthesis, resistance capacity for disease and cold, can protect roses against “little-leaf disease” and contribute the growth of rose buds. B plays an important role in the growth of root system, vegetative organs and reproductive organs (Wu, 2003).

The fact that as a regulator for plant growth, 5-ALA takes part in the chlorophyll synthesis is not new. It was found that 100~300 mg/L 5-ALA can, to some extent, contribute to the growth of carrots (Wang *et al*., 2004) and as a matter involved in the chlorophyll synthesis, 5-ALA can increase the net photosynthesis rate of plants (Liu *et al*., 2010). Previous studies have shown that foliar spraying of 800 times diluted 0.3 g/L 5-ALA would increase the biomass, unit area leaf, flower diameter, stem length and the unit area leaf chlorophyll content of roses and this is in accordance with the findings that 5-ALA can regulate the plant growth and take part in the chlorophyll synthesis (Hotta *et al*., 1998).

The results showed that spraying of 5-ALA singly or together with aqueous-organic solvents can both increase the unit area leaf chlorophyll content of roses, while the application of aqueous-organic solvents decreased its chlorophyll content. The underlying reason is that when used singly, 5-ALA dose not significantly contribute to the increase of rose unit area leaf and fresh leaf weight, while aqueous-organic solvents singly will enlarge the unit area leaf and increase fresh leaf weight significantly and the enlarged leaf consequently dilutes the chlorophyll content, which leads to the decrease of unit area leaf chlorophyll content in the treatment of aqueous-organic solvents. The unit rose leaf area and fresh weight in the treatment of 5-ALA and aqueous-organic solvent compounds are significantly higher than those treated by the aqueous-organic solvents singly and this will result in the relatively low increase in unit area leaf chlorophyll content compared with that treated by 5-ALA singly.

CONCLUSION

5-ALA and aqueous-organic solvents, either used singly or in combination, can improve the quality of the Rosa rugosa to some extent, with the latter having a better effect because the method can fully make use of the reciprocal interaction between 5-ALA and aqueous-organic solvents. As a consequence, it is an effective way to improve the quality of Rosa rugosa by foliar application of the new compound fertilizer with 5-ALA and aqueous-organic solvents. However, the problem is, despite its various strengths and the good improvement effect, foliar spraying could only serve as a supplementary way of fertilization with its limitation in fertilizer types and density and at the same time, constraints from the environmental conditions and features of the crop will prevent the rhizosphere nutrition of plants fully giving way to the exoroot nutrition.

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