ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

JASMONATE FOLIAR SPRAY INDUCED VEGETATIVE GROWTH AND PIGMENT COMPOSITION IN *Vigna radiata* L. WILCZEK

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Manuscript Info

Manuscript History:

Received: 22 November 2014
Final Accepted: 11 December 2014
Published Online: January 2015

Key words:

Vigna radiata, jasmonic acid, vegetative parameters, and biochemical constituents

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Abstract

The study was conducted to determine the effect of foliar Jasmonic acid (JA) application on vegetative growth and photosynthetic pigment composition of *Vigna radiata* (L.)Wilczek. JA was applied at five different concentrations (2ppm, 4ppm, 6ppm, 8ppm 10ppm) to 15 days old *Vigna* seedlings. Growth parameters such as shoot and root length, shoot and root fresh weight and dry weight and biochemical constituents such as chlorophyll a, b, total chlorophyll and carotenoid content were recorded in treated and control plants. All the five concentrations of JA enhanced the overall growth and biochemical constituents. The maximum increase in plant growth was found to be 8ppm. Application of JA also promoted the photosynthetic efficiency of *Vigna radiata* as evidenced in the photosynthetic pigment content. Compared to chlorophyll a, chlorophyll b was found to synthesize more which could be due to a change in the stoichiometry of PS II to PS I. Thus, the present work confirms the promontory effects of JA on overall growth and pigment constituents in *Vigna radiata*.

INTRODUCTION

Jasmonates are one of the recent plant growth regulators which cause decrease in damage due to environmental stresses in plants (Wang, 1999). Jasmonic methyl ester acid was discovered first as a matter of fragment from oil of *Jasmonium grandiflorum* (Sembdner and Parthier, 1993) and jasmonic acid was extracted from fungal culture in 1971. These compounds have been observed in plants like tea, rosemary and mint and some fungi (Creelman and Mullet, 1995). Pericarps, reproductive structure particularly ovary and the elongation zone of root and stem have a high amount of jasmonate. Jasmonates exist in 150 type including ferns, mosses and fungi and nearly 206 plant species have been identified to contain these compounds (Creelman and Mullet, 1997).

Methyl jasmonate (MeJA) and its free acid, jasmonic acid (JA), collectively referred to as jasmonates, are important cellular regulators involved in diverse developmental processes such as seed germination, root growth, fertility, fruit ripening, and senescence (Wasternack and Hause, 2002). In addition, jasmonates activate plant defense mechanisms in response to insect-driven wounding, various pathogens and environmental stresses, such as drought, low temperature, and salinity (Cheong and Choi, 2003).

Green gram (*Vigna radiata* L.Wilczek) is an excellent source of high quality protein. It also contains water soluble vitamins and minerals of dietary significance. Different plant growth regulators have been found to influence nutritional status of crop plants. An attempt has been made to study the impacts of jasmonate spray on growth, pigments composition and biochemical constituents of *Vigna radiata* .

MATERIAL AND METHODS

Plant material

Dry seeds of *Vigna radiata* (L.) Wilczek were procured from Agricultural Research Station, Kovilpatti, Tamilnadu,India and surface sterilized with 0.1% $HgCl_2$ for a minute and washed repeatedly with distilled water. Healthy seeds were selected and sown in pots containing mixture of red soil, black soil, and sand mixed in the ratio of 2:2:1. The seeds were allowed to germinate in dark for 48 hours. The percentage of seed germination was nearly 80%. Soon after emergence, the seedlings were shifted to daylight conditions and since the ambient climate was too hot for the seedlings, a 40% cut off mesh filter was used to surround the pots for an initial period of 2-3 days.

Jasmonic acid Foliar Spray

After a period of initial growth *Vigna radiata* seedlings were sprayed with five different concentrations of JA (2ppm, 4ppm, 6ppm, 8ppm, 10ppm) using an atomic sprayer. The seedlings were sprayed in JA until dropping. Each plant required about 10ml of spray solution. Jasmonic acid was purchased from Sigma Chemical Co.,U.S.A. JA solution was prepared by initially dissolving in 100µl of ethanol and concentrations of 5×10^{-6}M to 100×10^{-6}M (pH, 6.5) were made up with distilled water containing a wetting agent Tween-20 (Polyoxyethylene sorbitan monolaurate). Plants sprayed with 0.02% Tween-20 served as the control. The plants were arranged in a completely randomized design with three replicates (Khan et al., 2003). The foliar spray was given for three days early in the morning and growth analyses were done after 15 days of plant growth.

Determination of growth and photosynthetic pigment content

The morphological measurements such as, shoot and root length of JA treated and control plants were measured. The dry weight of shoot and root of the plants were estimated by incubating the material at 80^{o}C for overnight in a hot air oven. 50mg of fresh leaf tissues of control and treated plants were ground in 10ml of 100% acetone and the extract was centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant was measured at 662nm, 645nm and 470nm for Chl *a*, Chl *b* and carotenoids respectively using an ELICO SL-171 Spectrophotometer. The amount of Chl *a*, Chl *b*, total Chl and carotenoid content was calculated using the formulae of Wellburn and Lichtenthaler (1984).

Chl a (mg/l) $= (11.75 \times A_{662}) - (2.35 \times A_{645})$

Chl b (mg/l) $= (18.61 \times A_{645}) - (3.96 \times A_{662})$

Chl a + b (mg/l) $= (7.79 \times A_{662}) + (16.26 \times A_{645})$

Carotenoids (mg/l) $= \dfrac{1000 \times A_{470} - 2.27 \times Ca - 81.4 \times Cb}{227}$

(Ca refers to Chl a; Cb refers to Chl b)

Statistical Analysis

The experiments were performed in a randomized order. Data were expressed as means of three replicates with standard error. Statistical assays were carried out by one-way ANOVA using Tukey's test to evaluate whether the means were significantly different, taking $p<0.05$ as significant.

RESULTS AND DISCUSSION

Effect of JA on Vegetative Growth

Data presented in [Figure 1(a-f)] shows the variations in the vegetative growth induced by the foliar application of jasmonic acid at 2ppm, 4ppm, 6ppm, 8ppm and 10ppm in *Vigna radiata* . The morphological parameters included shoot and root length, shoot and root fresh weight, shoot and root dry weight. Regarding shoot and root length an appreciable increase upto 6% & 10% in shoot length at 6 and 8ppm and 13% &15% in root length was observed at 6 and 8ppm in 15 days old *Vigna* seedlings(Fig1. a&b).

Heil (2004) reported that shoot growth, fruit number, inflorescence number, and development rate of *Phaseolus lunatus* L. were enhanced by the application of 1 mM JA. These results are also inconsistent with Heijari et al.(2005) as seedling diameter, shoot fresh weight, root fresh weight and root length were hampered by MJ treatment in Scots pine.

The fresh weight of shoot and root was increased to 29% and 10% at 8 ppm of JA in *Vigna* (Fig1.c&d). The contribution of jasmonates to morphogenetic events has been well documented. Besides promoting cell

expansion, JA controls cell division and growth direction, supporting the adequate formation of tissues and organs (Koda, 1997).

The shoot dry weight got increased to 5% and 7% at 6 and 8ppm of JA treatment (Fig1.e).With regard to root dry weight a significantly increase upto 16% and 28% was observed after 4 and 6ppm of JA treatment respectively (Fig1. f) Maciejewska and Kopcewicz (2002) ascertained the contribution of jasmonate towards control of growth and elongation in shoot and root tissues, as well as in the formation of floral buds in *Pharbitis nil*. Thus foliar application of jasmonic acid at 8ppm was effective in improving growth parameters of *Vigna radiata*.

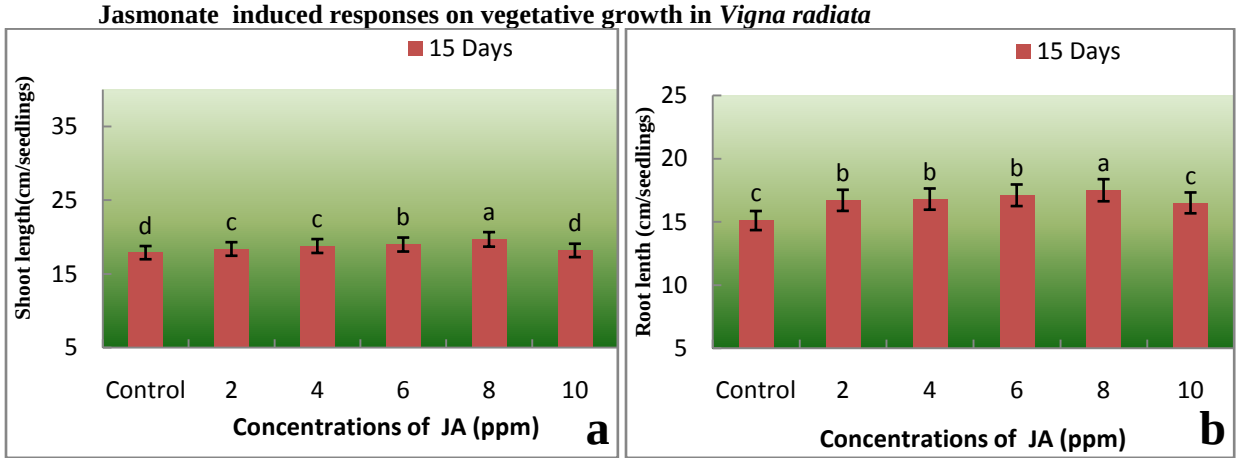
On unit fresh weight basis to photosynthetic pigment composition [Fig.2 (a-d)] foliar application of jasmonic acid at 8ppm enhanced accumulation of chlorophyll a and b, total chlorophyll and carotenoids compared to control. Maximum increase in chlorophyll a content 5% and 7% was observed at 6 and 8ppm of JA concentration in 15 days old *Vigna radiata* seedlings (Fig. 2 a). Chlorophyll *b* content was significantly increased to 10% at 8ppm when compare with control plants (Figure 2 b). The percentage of increase was much apparent in Chl *a* content than Chl *b* (Fig. 2 a& b).

Jasmonic acid and its related compounds have been shown to stimulate the accumulation of plant pigments. Jasmonates, inducing chlorophyll accumulation, have already been reported to be effective in greening cucumber cotyledons (Fletcher et al., 1983) and *Chlorella vulgaris* (Czerpak et al., 2006).

Maximum increase in total chlorophyll content was observed at 6 and 8ppm of JA concentration, nearly 5% and 8% increase was noticed in 15 days old *Vigna radiata* seedlings (Fig. 2 c). It seems that the stimulatory effect of JA on photosynthetic pigment accumulation could be due to strong effect on chlorophyll synthetic pathway specially δ-ALA (aminolevulinic acid) which is the rate limiting step in the biosynthesis of chlorophyll during earliest stages of greening (Beale, 1978).

The changes in carotenoids level under JA treatment at 8ppm was significantly increased to 30%, as noticed in 15 days *Vigna* seedlings respectively (Fig.2 d). Almost all the JA concentrations favored the pigment increase (Fig.2 a-d). Kovac et al. (1994) reported that JA treatment resulted in an increase of active cytokinin concentration with enhanced chlorophyll accumulation.

Jasmonate induced responses on vegetative growth in *Vigna radiata*



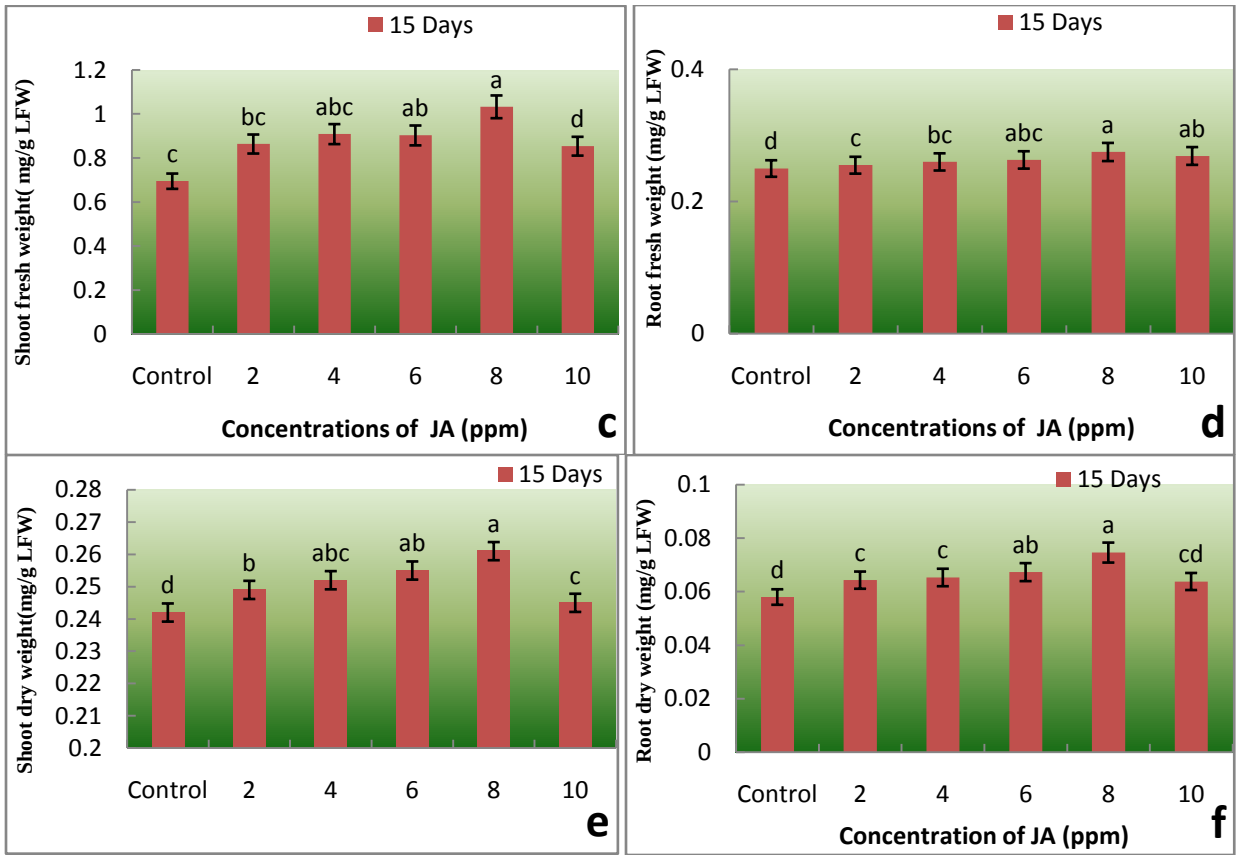
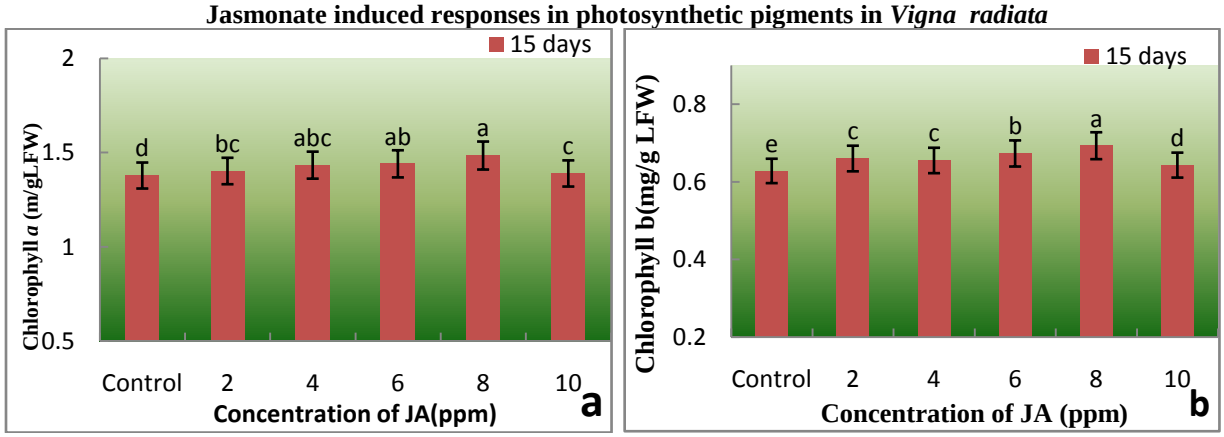


Fig.1 (a,b,c,d,e,f). Effect of Jasmonate spray on growth parameters in intact seedlings of *Vigna radiata* (L.) Wilczek. The concentrations of JA were 2, 4, 6, 8, 10ppm. Each seedling received 10ml JA spray given during the early hours of the day after 15 days of growth. The measurements were made in 15 days old seedlings. Each value represent the mean of six independent measurements (Mean ±SE, n=6). Bars carrying different letters are significantly different at p< 0.05.

Jasmonate induced responses in photosynthetic pigments in *Vigna radiata*



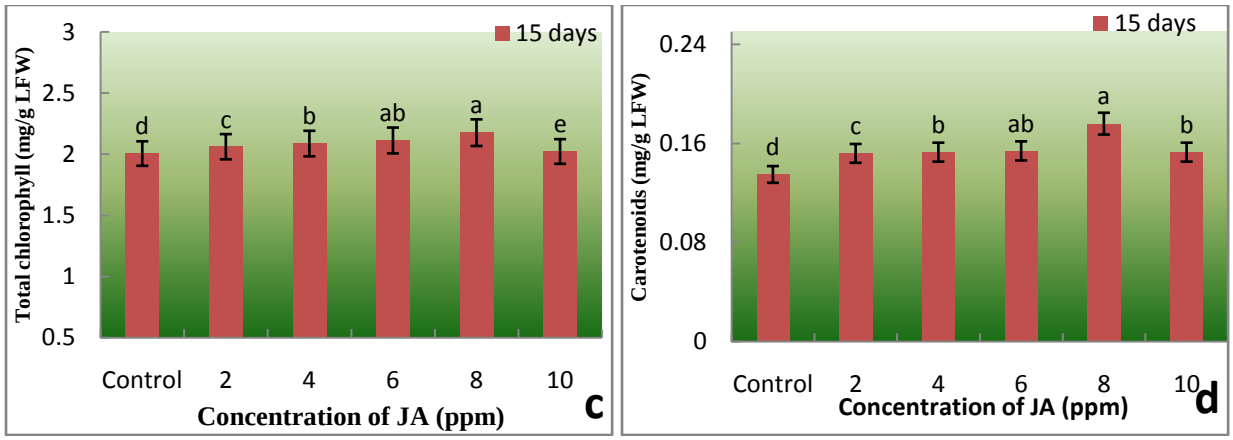


Fig.2 (a,b,c,d). Effect of Jasmonate spray on photosynthetic pigment composition in intact seedlings of *Vigna radiata* (L.) Wilczek. The concentrations of JA were 2, 4, 6, 8, 10ppm. Each seedling received 10ml JA spray given during the early hours of the day after 15 days of growth. The measurements were made in 15 days old seedlings. Each value represent the mean of three independent measurements (Mean ±SE, n=3). Bars carrying different letters are significantly different at p< 0.05.

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