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Research Paper

EFFECT OF 24-EPIBRASSINOLIDE SPRAY ON VEGETATIVE GROWTH, PIGMENT COMPOSITION AND BIOCHEMICAL CONSTITUENTS OF *Vigna mungo* (L.) HEPPER (BLACKGRAM)

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Abstract

In the present experiment, an attempt has been made to understand the 24-epiBrassinolide (eBR) induced responses in *Vigna*. Foliar spray of eBR at 0.5 µM to 2.0 µM concentrations significantly enhanced the rate of vegetative growth, photosynthetic, non-photosynthetic pigments and biochemical constitutions. Among the concentrations 1.0 µM was found to be useful in triggering the growth responses. Thus the exogenous application of BR proved to be physiologically and biochemically efficient in improving the vegetative growth of *Vigna*.

Key words: chlorophyll, foliar, flavonoids, proline, *Vigna*.

INTRODUCTION

Plant growth regulators are being increasingly employed to improve rooting and vegetative propagation of plants and helps in quick multiplication of such plants and increase roots availability [1]. Brassinosteroids are a new group of phytohormones with significant growth promoting activity and are essential for many processes in growth and development [2] and [3]. Brassinosteroids were isolated from pollen extract of *Brassica napus* [4] because of their growth promoting properties and their potential use for enhancing crop production. Exogenous application of BRs affects a broad spectrum of physiological responses like cell expansion, vascular differentiation, reproductive development, seed germination, flowering, and fruit set in plants [5]. Apart from growth promoting effects, BRs are also reported to confer resistance to plants against various abiotic and biotic stresses like heat, drought, heavy metals, infections, pesticides, salt, and even viruses [6,7,8]. In the present study the effect of eBR on vegetative growth and photosynthetic and biochemical constitutions has been studied in *Vigna mungo* (L.) Hepper.

MATERIAL AND METHODS

Healthy and uniform seeds of *Vigna mungo* were purchased from Agricultural Research Institute, Kovilpatti. The percentage of seed germination was found to be 85%. The seeds were sown in pots containing mixture of red soil, black soil, and sand mixed in the ratio of 2: 2: 1. Soon after emergence of the cotyledons, the seedlings were shifted to daylight conditions. 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.

Foliar spray Treatment

Brassinosteroid (eBR) was obtained from Mount Biosciences technology and solutions (Hyderabad) and initially dissolved in 100µl of methanol and concentrations of 0.5×10^{-6} M to 2.0 $x10^{-6}$M were made up using distilled water. The seedlings were sprayed with solutions using an atomic sprayer. Each seedling was sprayed with 5ml of BR solution. The foliar spray was given during the early hours of the day and continued for three days. The analyses were carried out only in 10 and 15 days old seedlings.

Estimation of pigments

The procedure of extracting of photosynthetic pigments from freshly harvested leaves was the same as that of [9]. The amount of Chl *a*, Chl *b*, total Chlorophyll and carotenoids was measured at 662, 645 and 470 nm respectively using a Hitachi U-200 double beam spectrophotometer and estimated using the formulae of [10].

Estimation of soluble protein, proline and *in vivo* NR Activity

The leaves were homogenized in ice-cold 0.1mM phosphate buffer (pH 6.8). The homogenate was centrifuged at 10,000 rpm for 10min at 4^0C and the supernatant as used to determine soluble protein content. Protein content was determined following the method of [11] having bovein serum albumin as standard. The Proline content in fresh leaf samples was determined following the method of [12]. *In vivo* NR activity was measured following the method of [13].

Flavonoids and anthocyanins

Fresh leaf bits were incubated in 80% acidified methanol (methanol:water:HCl; 80:20:1) for 12 h at 4°C in dark to extract the flavonoids with intermittent shaking. The absorbance of the methanol extract at 315 nm was used to quantify the flavonoid content [14]. Anthocyanins were extracted by grinding the leaves in 80% acidified methanol and the clear extract was used to estimate the anthocyanin concentration by measuring the absorbance at 530 and 657 nm [15].

Statistical Analysis

The results were expressed as arithmetic mean $\pm$ standard error (SE). Group difference was tested by one-way analysis of variances (ANOVA). Multiple comparisons were made using online Tukey test. All statistical calculations were performed using online statistical tool Vassarstats.net. The level of significance was expressed as $P < 0.05$.

RESULTS

The vegetative growth parameters include shoot length, root length, shoot fresh weight, shoot dry weight and leaf area. Different concentrations such as 0.5 to 2.0 µM were sprayed on to the intact 5 day, 10, 15 and 20 days old seedlings. Among the concentrations tested, 1 µM concentration of eBR was found to increase almost all the growth parameters after 5 days of growth (Fig.1). Similar positive response was noticed with increase in hormone concentration at different stages of plant growth. On a unit fresh weight basis, a uniform increase in Chl *a*, *b* and carotenoid content was observed. With respect to Chl *a,* Chl *b* was found to accumulate more in terms of fresh weight (Fig. 1-2). The photoprotective pigment namely carotenoids show parallel increase to Chl. Thus, the optimal concentration for maximum induction of plant height as well as photosynthetic pigment content was found to be 1.0µM of eBR.

The expression of hormonal response was also witnessed in the level of soluble protein and proline content. Leaf soluble proteins were quantified as per the standard procedures and maximum level was noticed at 1.0µM eBR spray (Fig.2). Unlike other biochemical constituents, the stress osmolyte namely proline was not triggered up at hormone concentrations (Fig.2). The level of proline in *Vigna* seedlings after hormone treatment is an indication to show that the application of eBR to intact plants was not stressful. Examination of non-photosynthetic pigments such as anthocyanin and flavonoids were not significantly induced upon hormone treatment. Both the pigments are known to protect plants against abiotic stresses. In the

present experiment, eBR spray had not induced significant changes in the content of non-photosynthetic pigments (Fig.2).

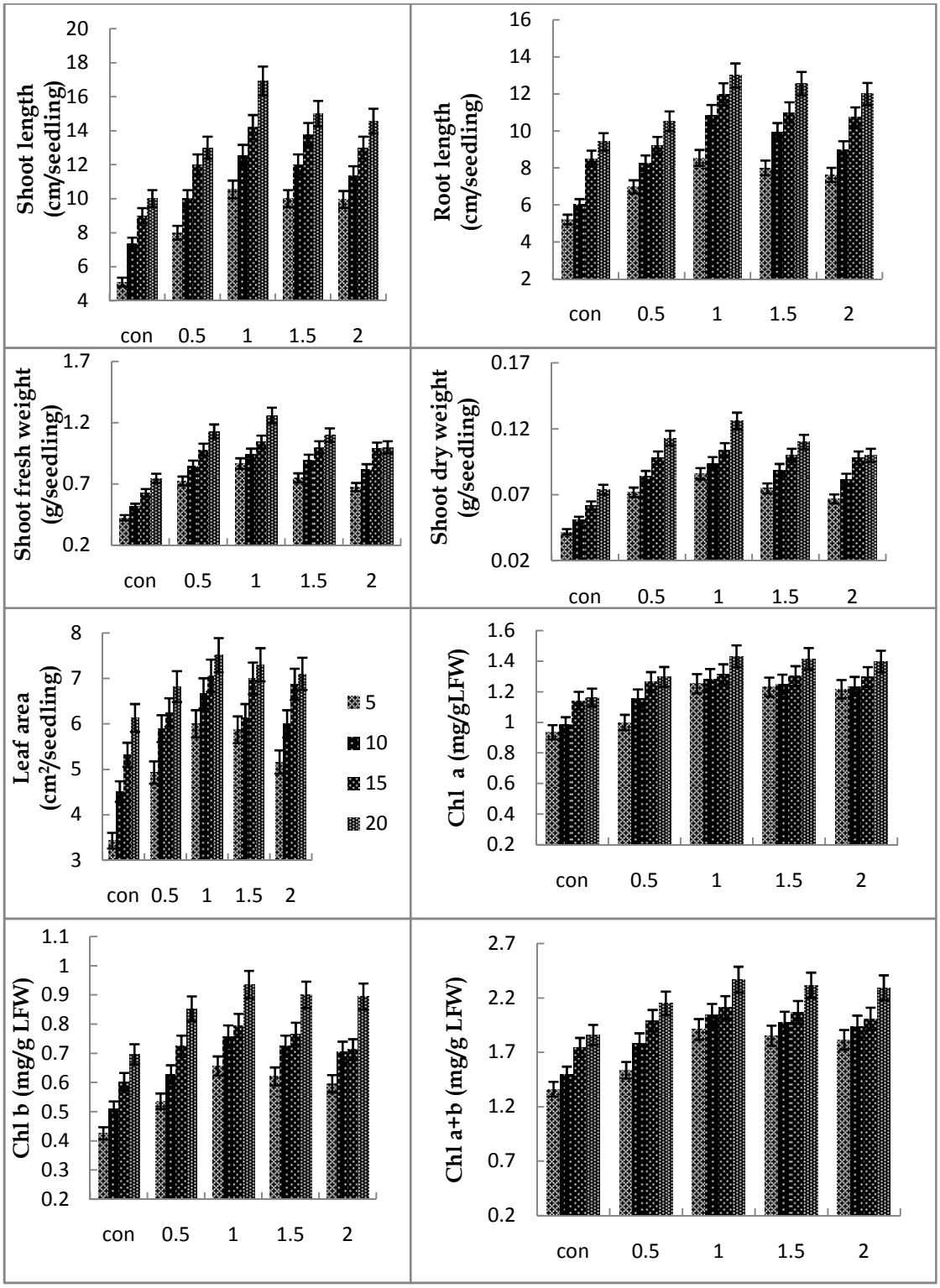

Fig. 1 Changes in morphological and photosynthetic parameters of *V.mungo* treated with various concentration of 24-eBRL (Shoot length, Root length, Shoot fresh weight, Shoot dry weight and Leaf area). The values represent an average of 5 independent measurements.

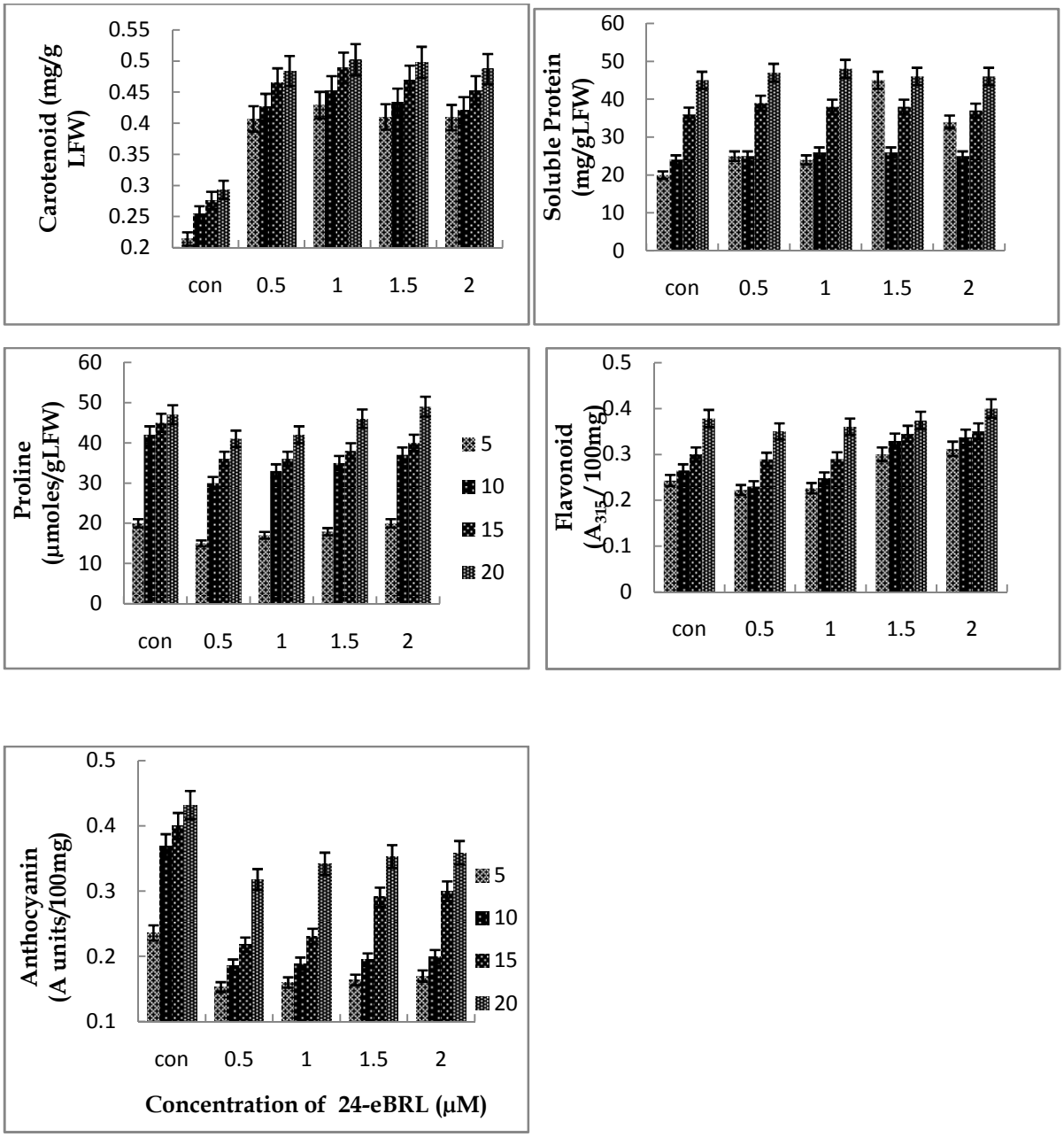

Fig. 2 Changes of biochemical and non-photosynthetic pigment of *V.mungo* treated with various concentration of 24-eBRL (soluble protein, proline, Flavonoid and Anthocyanin). The values represent an average of 3 independent measurements.

DISCUSSION

In the present studies, BR effectively stimulated the elongation and formation of lateral shoots and shoot buds. Similar results were reported by [16] in *Eucalyptus* and *Malus prunifolia*. Increase in Chl content under BR treatment was reported in groundnut by [17]. [18] stated that, descending epi-BL concentrations resulted in marked changes in pigment contents of different parts of the soyabean seedlings grown in dark and light. Anthocyanin amount was found to remain unchanged by descending epi-BL concentrations in the control cotyledons of the seedlings grown in light while it increased adversely in hypocotyls with applied concentrations.

The application of epi-BL protect plants against UV radiation by increasing protective pigments such as flavonoids and anthocyanins which could be generated by polyphenols synthesis pathway [19]. *Vigna* seedlings sprayed with 24epi-BL increased the protein and NR activity in 5, 10 , 15 and 20 d- old seedlings except proline content. Similar increase in total

protein level was reported by [20] and [21]. Stimulation of NR activity in black gram by BR treatment was reported by [22]. Phenolic compounds play paramount role in nitrogen metabolism of plants. Brassinolides and, if endogenous Brassinolides are directly involved in the control of cell expansion, they must be present in such tissue. Approaches to establishing this include the analysis of levels in a Brassinolide-sensitive zone of pea stem [23] and localization of an exogenously supplied 125I-BR, which accumulated in the elongating zone of mung bean epicotyls and the apex of cucumber seedlings [24]. This experiment on the chemistry and morpho-physiology of Brassinolides provides a convincing body of evidence that these plant steroids in minute quantities are essential regulators of plant growth and development.

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