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Effect of 24-Epibrassinolide induced changes in Seed germination, Growth and Biochemical Composition in Early seedling stages of *Brassica juncea* (L.) Czernj

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Abstract

Brassinosteroid (BR) is the endogenous plant growth regulator involved in various physiological processes of plant growth and development. In the present experiment, an attempt has been made to understand the 24-epiBrassinolide (eBR) induced responses in *Brassica juncea*. Application of eBR at 1µM and1.5µM significantly enhanced the rate of *Brassica* seed germination to a level of 92% and 94% respectively in *Brassica*. Dark incubated *Brassica* seedlings behaved differently to the application of BR. The internode length rather than root length was significantly increased upon eBR treatment. Among the concentrations, only 1.0 µM and 1.5 µM were found to be useful in triggering the growth responses. With increase in time, growth and biochemical parameters such as soluble protein, glucose content and β- amylase activity were also increased under BR treatment. Thus the exogenous application of BR proved to be physiologically and biochemically efficient in improving the vegetative growth of *Brassica*.

Key-Words: *Brassica*/ glucose/ internode length/ β-amylase / seed germination

Introduction

In addition to the conventional plant hormones such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene, Brassinosteroids are naturally occurring plant growth regulators recognized as a sixth class of plant hormones (Mandava *et al*.,1987). The BRs were first explored nearly 40 years ago, when Mitchell *et al*. (1970) reported promotion in stem elongation and cell division by the treatment of organic extracts of rape seed (*Brassica napus*) pollen. United States Department of Agriculture (USDA) group mounted a major effort to identify the active constituent in brassins. BRs are important plant growth regulators in multiple developmental processes at nanomolar to micromolar concentrations.

BRs also influence various other developmental processes like germination of seeds, rhizogenesis, flowering, senescence, abscission and maturation. They also confer resistance to plants against various abiotic and biotic stresses (Mussig, 2005; Sasse, 2003; Syed Ali Fathima *et al* 2011; Yokota, 1999). The present study was aimed to examine the influence of BRs on seed germination and seedling growth and biochemical characteristics in early seedling stages of *Brassica juncea* (L.) Czernj.

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Material and Methods

Healthy and viable seeds of *Brassica juncea* (L.) Czernj. were procured from Agricultural Research Centre, Kovilpatti, Tuticorin district. 24-epiBrassinolide (24-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 with distilled water. The seeds were soaked in 10ml of BR solutions (0.5 to 2.0µM) for different time interval like 12h, 24h, 48h, and 72h. The percentage of seed germination was nearly 85%. Ten seedlings per treatment were analyzed for the changes in internode length and root length. The distance from the region of contact to the cotton mat to the apex was measured and expressed as internode length and root length. Protein content was estimated by Lowry's (1951) method using Bovine serum albumin as standard.

β amylase activity was estimated by Peter Bernfield's (1955) method. The rate of β-amylase activity was determined by measuring the amount of maltose formed per unit time per fresh mass. The total glucose content was estimated using Anthrone reagent (Jayaraman, 1981).

Results and Discussion

Seed germination

The percentage of germination was calculated from 12h of incubation of seeds in various concentrations of BR. The percentage of germination increased to 92% and 94% at 1μM and 1.5μM concentration of BR was shown in Fig.1. Sharma and Bhardwaj (2007) reported that the germination percentage of 7-d-old *Brassica* seedlings was significantly increased due to the application of 24-epiBL especially at low concentrations (10^{-9} M and 10^{-11} M). Hayat and Ahmad (2003) found that homobrassinolide increased the germination percentage by 17% in wheat grains. Similarly, shoot and root lengths and their fresh weights were increased by the application of BRs. Brassinosteroids had profound effect on rice germination and at seedling stage (Anuradha and Rao, 2001).

BR induced increase was also noted on the internode length. The increase is shown in Fig.2. Both 1 and 1.5μM concentrations proved to be best. Yin *et al.* (2002) proposed that BRs promote stem elongation by regulating gene expression and by enhancing BRs signal through a plasma membrane localized receptor kinase BR I1. Beneficial effects of BR on shoot length have also been reported by various workers (Shen, 1988; Sasse, 1991). With regard to root length, the percentage increase was 44% and 88% respectively at 1 and 1.5 μM of eBR after 72h of incubation (Fig.2).

The effects of BRs on root growth were comparable to those of Sathiyamoorthy and Nakamura (1990) and Romani *et al.* (1983) whereas contradictory to those of Roddick and Ikekawa (1992). Similarly, the percentage of glucose content upon BR treatment also followed an increasing trend as 32% at 1.0μM after 12 h and 45% at 1.5μM after 48h (Fig.2). In rice, brassinolide decreased the starch content in leaf sheaths and culms whereas increased the content of both in hulled grains. The increase might be due to enhanced photosynthetic capacity of the plants as influenced by the 28-homoBL and 24-epiBL application (Braun and Wild, 1984). The positive impact of the BRs on the production and metabolism of carbohydrates is quite well known in plants (Yu *et al*., 2004; Vardhini *et* al., 2011). The level of soluble protein content also increased upon BR treatment during different time intervals. The impact was more at 1 and 1.5 μM of BR. Nearly 27% at 1μM and 50% at 1.5μM was observed after 12h (Fig. 2).

The effect of eBR was analyzed on the β-Amylase activity in mustard germinating seeds. The enzyme activity would represent the amount of maltose formed per unit time and mass. The percentage of increase in β- amylase activity was 17% at 1.5μM of eBR after 48h as compared to control (Fig.2). Similar homobrassinolide (HBR) induced seed germination was reported by Chang and Cai (1998) and Dong *et al*. (1989). These results show that HBR not only increased germination and seedling growth but also help in stress tolerance (Dong *et al*., 1989). Thus, in the present study 24-epibrassinolide was found to promote the seed germination, internode length, root length, glucose, protein content and β- amylase activity in *Brassica juncea* even at a low (1.5μM) concentration.

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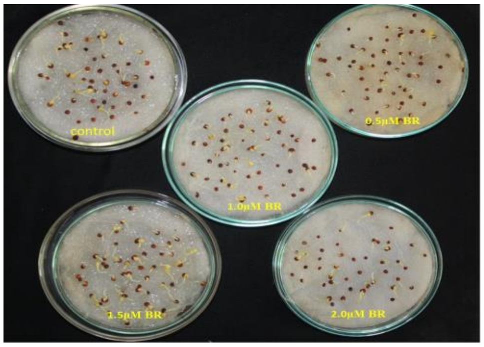

Fig.1: Changes in seed germination of *Brassica* seeds in various concentrations of eBR soaked for 24 h dark

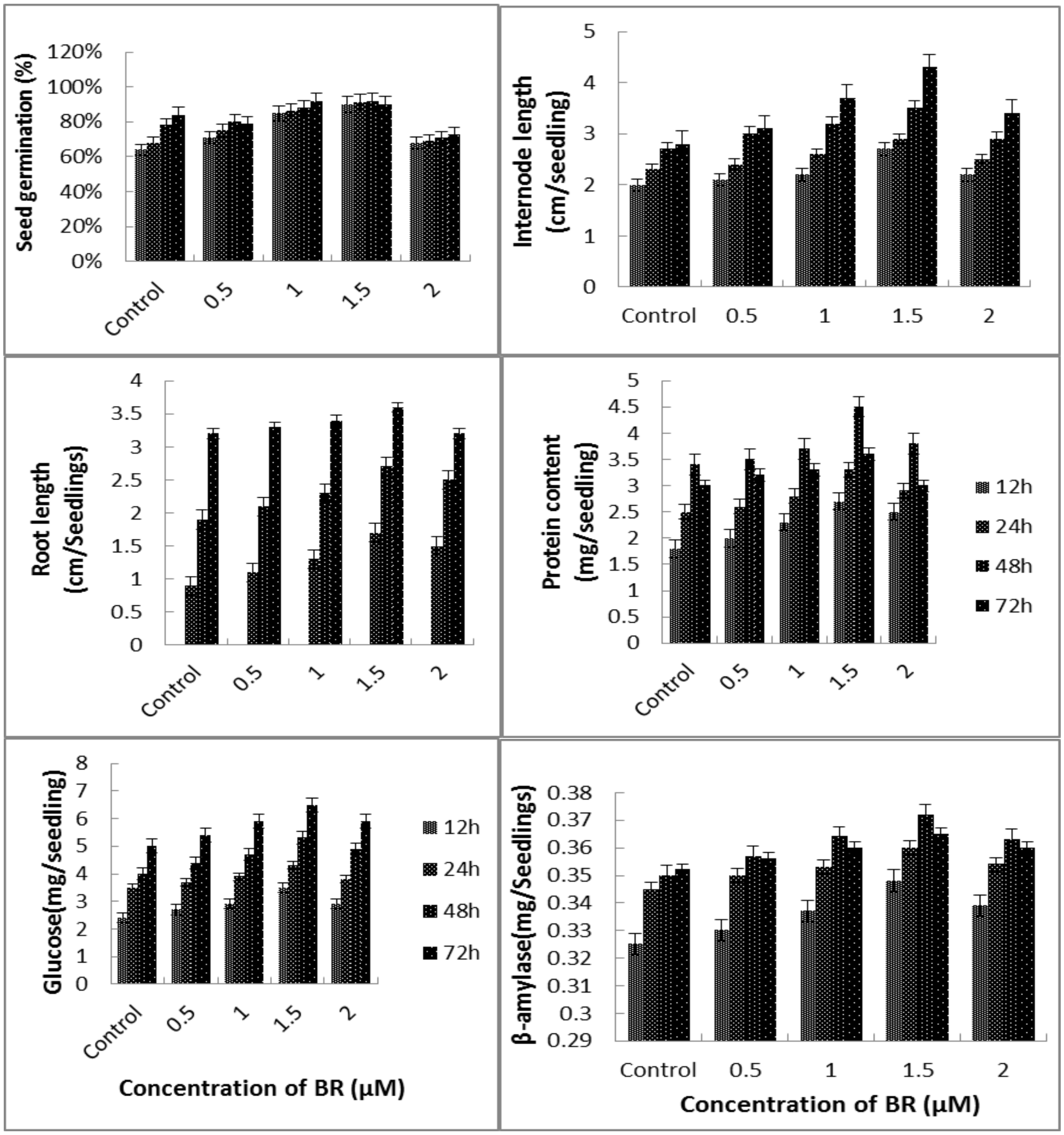

Fig. 2: Changes in morphological and biochemical parameters of *Brassica*seedlings measured at different time intervals. The seeds were soaked in various concentrations of eBR in dark for 24 h. The values are an average of three independent measurements. Mean ± SE, n=5

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