Effect on morphological characters in *Eucalyptus tereticornis* Sm. at juvenile stage under the influence of moisture stress and mitigation by Brassionosteroids and sucrose

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Abstract

An experiment was carried out to study the effects of brassinosteroids and sucrose under moisture on the morphological of *Eucalyptus tereticornis* Sm. at juvenile stage under controlled condition in nursery. The experiment was conducted with the aim of investigating the study the effect of moisture stress levels on growth and physiological characters also to establish the effective combination of BRs and sucrose to combat moisture stress. The study was carried out on the clonal seedlings P-23 of *Eucalyptus tereticornis* at the Department of Forestry, CCSHAU, Hisar. The clonal seedlings were planted in pots in nursery containing sand and soil in 2:1 proportion. Mineral composition of the pot soil was assessed before planting the *Eucalyptus tereticornis* plants. All the pots were received normal watering till 90 days of seedling growth. Three months old potted seedlings were subjected to moisture stress (M1 to M3) by withholding water. The different levels of brassinosteroids (0, 5, 10, 15 and 20 ppm) and sucrose (0, 1, 3 and 5% (w/v)) were applied to the seedlings. It was observed that increment in shoot length, root length, fresh and dry biomass of fine root, coarse root and total plant biomass significantly declined under moisture stress. But in case of the root length, fresh and dry weight of fine root and coarse root increased and total plant fresh and dry weight decreased with increased the moisture stress levels (M1 to M3).

Keywords: Brassionosteroids, growth, moisture, sucrose

Introduction

*Eucalyptus tereticornis* popularly called the Mysore gum belongs to family Myrtaceae, is an industrially and economically important fast-growing multipurpose tree species. Though of Australian origin, in India, the Eucalyptus was first introduced by Tipu Sultan between 1782-1790 in Nandi Hills. It is also grown in countries like Argentina, Bangladesh, Brazil, Indonesia, South Africa, Philippines etc. It can grow on a variety of soils, with a preference for deep, well drained soils of fairly light texture, including alluvial soils, silts and clays. The most important characteristics contributing to its popularity under Indian condition are: it is fast growing, capable of over topping weeds, coppices well, is fine hardy, browse resistant and it has the ability to adapt to a wide range of edapho-climatic conditions.

One of the major factors causing decline in the productivity of the agricultural produce is “stress”. An environmental factor that limits the crop productivity is referred to as stress or disturbance. Plants are often exposed to environmental stresses such as influence of abiotic stresses like moisture stress. Drought is one of the most important abiotic stress factors limiting plant growth and productivity (Polle et al., 2006). During water stress, soil water is more strongly retained and solute transfer to plants is less efficient and may not meet nutrient demands of the plants. The response of plants to water stress depends on several factors such as developmental stage, severity and duration of stress. Water stress in plant affects many important physiological processes, which counts much to the growth and total production of almost all the tree species. It is also expected that one of the consequences of global climate change will be increased periods of drought in many areas world-wide (Solomon et al. 2007) [16]. Water stress is characterized by a reduction of water content and leaf water potential, closure of stomata, and decreased growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism, and finally the death of plant (Jaleel et al. 2008) [7].
Brassinosteroids (BRs) are polyhydroxylated steroidal phytohormones, which play an important role in regulation of various plant growth and developmental processes, e.g. stem and root growth, floral initiation, and the development of flowers and fruits (Hayat and Ahmad, 2003) (9) and (Sasse, 2003) (12). Mitchell et al. (1970) (9) discovered BRs which were later extracted from the pollen of Brassica napus by Grove et al., (1979) (4). To date, more than 70 BR-related phytohormones have been identified in plants (Zhao and Li, 2012) (20). BRs increase adaptation to various abiotic stresses such as drought (Anjum et al. 2011), (Mahesh et al., 2013), and heavy metal stress (Yusuf et al., 2011, and Yusuf et al., 2012) (19, 18). BRs have been found in a wide range of plant species including higher plants and lower plants. BRs have been detected in various plant parts including pollens, seeds, leaves, stem, roots and flowers. One of the most promising roles of BRs is their ability to confer resistance to wide array of abiotic stresses (Bajguz and Hayat, 2009) (2). BRs assumed to function as a “master switch” in abiotic and biotic responses (Bajguz, 2010) (1). Soluble sugars, especially sucrose play an obviously central role in plant structure and metabolism at the cellular and whole-organism levels. They are involved in the responses to a number of stresses, and they act as nutrient and metabolite signalling molecules that activate specific or hormone cross talk transduction pathways, thus resulting in important modifications of gene expression and proteomic patterns (Hirabayashi, 1996) (6). Sucrose also plays an important role in development of anti-oxidative systems such as SOD, POD in plants under drought stress condition Ślesak et al., 2006 (15).

In most tropical countries such as India, frequent drought of varying intensity and duration due to unpredictable and low rainfall is very common. Also, the current climatic projections predict not only increasing air temperatures but side by side a rise in frequency and duration of intensive summer drought. These stresses in plants, affect many important physiological processes and ultimately the forest yield. Tremendous efforts have been taken on improving morphological and physiological traits of nursery-grown seedlings as well as improving genetic quality of seedlings that may requires a long-term strategy of seed selection. Applications of BRs and sucrose in Eucalypts are amongst that may improve growth under moisture stress conditions. The systematic studies on the effect of brassinosteroids and sucrose on morphological characters under moisture stress conditions are scanty in this versatile species. Therefore, the present investigation has been planned to know how these stresses affect the plant physiological system, and to find out where ameliorative approaches are needed.

**Materials and Methods**

The study was carried out on the clonal seedlings P-23 of *Eucalyptus tereticornis* at the Department of Forestry, CCSHAU, Hisar. The clonal seedlings were planted in pots in nursery containing sand and soil in 2:1 proportion. Mineral composition of the pot soil was assessed before planting the *Eucalyptus tereticornis* plants. All the pots were received normal watering till 90 days of seedling growth. Three months old potted seedlings were subjected to moisture stress by withholding water as per the requirement of treatment mentioned in below in head (irrigation). The different levels of brassinosteroids (0, 5, 10, 15 and 20 ppm) and sucrose (0, 1, 3 and 5% (w/v) were applied to the seedlings.

**Irrigation**

Four moisture levels were selected by preliminary test basis. Irrigations were given to the pots according to the need of experiment as:

1. 1st moisture level (M0) (100% field capacity) - Every pot was irrigated by 2050 ml of water to obtain field capacity.
2. 2nd Moisture level (M1) (80% of the field capacity) - Every pot was irrigated by 1640 ml of to obtain 80% of the field capacity.
3. 3rd Moisture level (M2) (60% of the field capacity) - Every pot was irrigated by 1230 ml of water to obtain 60% of the field capacity.
4. 4th Moisture level (M3) (40% of the field capacity) - Every pot was irrigated by 820 ml of water to obtain 40% of the field capacity.
5. To maintain the moisture levels, daily evapotranspiration was recorded and accordingly irrigation of the pots was done with the tap water.

**Morphological characters**

Two plants from each treatment (two replicates) were undertaken to determine the shoot length, root length, fresh and dry weight of plant. Shoot length was measured in cm from the basal region to the tip of the stem, root length was also measured in cm but only after final harvest from collar region to the tip of root, fresh weight of coarse root, fine root and total plant (g) of the seedlings were determined at harvest and dry weights (g) were also determined after drying them in oven at 60°C for one week.

**Results and Discussion**

Moisture stress causes the deleterious effect on plants by disturbing their metabolism that ultimately affects the growth, physiological and biochemical directly or indirectly. A thorough perusal of the literature showed that paucity of well-maintained work on brassinosteroids and sucrose the physio-biochemical responses of *Eucalyptus tereticornis*. Sm. to moisture stress. Successful attempts were also made under moisture stress by application of BRs and sucrose that induce tolerance mechanism in eucalyptus seedling. In the present study, an attempt has been made to obtain basic information, which may prove beneficial and can be further exploited at growth and physiological basis for the improvement of *Eucalyptus tereticornis* seedling for moisture tolerance. The results presented under the experimental findings are being discussed here with the support of the published literature available on the subject. The results obtained in the present investigation are discussed in the light of available literature with the following head:

**Morphological characters**

Morphological characters are influenced by various factors besides its genetic makeup and for assessing crop productivity in various crops, it is an important tool. The pot culture studies revealed that decline in growth of *Eucalyptus tereticornis* seedlings occurred in response to increased moisture stress concentrations. Moisture stress caused significant decreases in periodic increment (%) in shoot length (Table 4) as compared to control plants. Similar results were also found by Christina et al., (2015) (1) that shoot length was significantly declined under the moisture stress in *Eucalyptus grandis*. It was also observed in the present study that root length was significantly declined under moisture...
stress application, but in case of the root length increased with increased with increases the moisture stress level from M0 to M3 as compared to control. Therefore, fresh and dry biomass of fine root and coarse root significantly increased but total plant fresh and dry biomass significantly decreased under the moisture stress. The finding of this study is supported by Susiluoto and Berninger (2007) \(^{17}\) in Eucalyptus microtheca seedlings under the moisture stress treatment.

The present investigation revealed the periodic increment in shoot length (%), total plant fresh and dry weight were significantly reduced as shown in Table 1, Table (3) and (Table 4) from 35.69 to 12.53%, 52.14 to 19.46 g and 16.36 to 6.11 g, respectively when the levels of BRs concentration in ppm: BRs = (0, 5, 10, 15, 20) and Su=(0, 1, 3, 5). M0, M1, M2 and M3 as compared to control. The interactive effect of BRs × Su was found maximum under each levels of moisture stress. Maximum root length [6.05 cm (control), 7.13 cm (M3)] was observed with the combined application of BRs (20 ppm) × Su (5 ppm). Similarly, Singh et al. (2008) observed an increase in shoot length of wheat plant by foliar spray of BRs in maize under moisture stress. Sharma (2016) also found that fresh and dry matter and root and shoot length in tomato was reduced under the moisture stress condition which was significantly increased by the foliar application of Brassonsteroids in tomato plants.

| Table 1: Periodic increase in shoot length (%) under the influence of moisture stress at various protective applications of BRs and Su |
|---|
| **Treatments** | **M0** | **M1** | **M2** | **M3** |
| | Su 0 | Su 1 | Su 3 | Su 5 | Mean | Su 0 | Su 1 | Su 3 | Su 5 | Mean | Su 0 | Su 1 | Su 3 | Su 5 | Mean |
| BRs 0 | 35.69 ±5.75 | 37.58 ±5.63 | 39.15 ±5.60 | 40.69 ±4.72 | 38.28 ±4.67 | 29.70 ±3.51 | 30.98 ±3.51 | 31.60 ±3.51 | 32.22 ±3.51 | 31.13 ±3.51 | 23.44 ±4.77 | 24.93 ±5.75 | 25.66 ±5.63 | 26.38 ±5.60 | 25.10 ±5.57 |
| BRs 5 | 46.95 ±6.74 | 47.41 ±5.45 | 48.10 ±5.23 | 48.80 ±5.23 | 47.82 ±4.94 | 34.72 ±3.91 | 34.91 ±3.91 | 35.18 ±4.48 | 35.47 ±5.16 | 35.07 ±5.16 | 29.30 ±4.94 | 29.52 ±5.63 | 29.84 ±5.63 | 30.17 ±5.63 | 29.71 ±5.63 |
| BRs 10 | 49.57 ±5.03 | 50.43 ±6.01 | 51.13 ±5.16 | 51.83 ±5.16 | 50.84 ±4.94 | 35.93 ±3.62 | 36.12 ±3.62 | 36.40 ±4.23 | 36.67 ±4.23 | 36.28 ±4.23 | 30.71 ±5.75 | 30.93 ±6.23 | 31.25 ±6.23 | 31.58 ±6.23 | 31.52 ±6.23 |
| BRs 15 | 52.98 ±6.50 | 53.46 ±5.14 | 54.15 ±5.14 | 54.85 ±4.94 | 53.86 ±4.23 | 37.14 ±3.99 | 37.33 ±3.99 | 37.60 ±4.19 | 37.88 ±4.19 | 37.49 ±4.19 | 32.12 ±4.77 | 32.34 ±5.21 | 32.66 ±5.21 | 33.09 ±5.21 | 32.53 ±5.21 |
| BRs 20 | 56.02 ±5.54 | 56.51 ±5.54 | 57.17 ±5.54 | 57.84 ±5.18 | 56.89 ±4.48 | 38.35 ±5.54 | 38.55 ±5.54 | 38.81 ±4.48 | 38.98 ±4.48 | 38.79 ±5.54 | 35.54 ±6.71 | 35.76 ±6.71 | 36.09 ±6.71 | 36.44 ±6.71 | 36.25 ±6.71 |
| Mean | 48.32 ±4.81 | 49.08 ±4.81 | 49.94 ±4.81 | 50.80 ±4.86 | 49.54 ±4.86 | 35.17 ±3.82 | 35.58 ±3.82 | 35.92 ±3.82 | 36.26 ±3.82 | 35.73 ±3.82 | 29.82 ±4.87 | 30.30 ±4.87 | 30.70 ±4.87 | 31.10 ±4.87 | 30.48 ±4.87 |

**Values presented are means (±SE, n = 80).**

| Table 2: Effect on root length under the influence of moisture stress at various protective applications of BRs and Su |
|---|
| **Treatments** | **M0** | **M1** | **M2** | **M3** |
| | Su 0 | Su 1 | Su 3 | Su 5 |
| BRs 0 | 5.51 ±0.63 | 5.54 ±0.63 | 5.57 ±0.63 | 5.54 ±0.63 |
| BRs 5 | 5.63 ±0.63 | 5.64 ±0.63 | 5.67 ±0.63 | 5.69 ±0.63 |
| BRs 10 | 5.75 ±0.63 | 5.76 ±0.63 | 5.78 ±0.63 | 5.80 ±0.63 |
| BRs 15 | 5.87 ±0.63 | 5.88 ±0.63 | 5.90 ±0.63 | 5.92 ±0.63 |
| BRs 20 | 5.99 ±0.63 | 6.00 ±0.63 | 6.02 ±0.63 | 6.05 ±0.63 |
| Mean | 5.75 ±0.63 | 5.76 ±0.63 | 5.78 ±0.63 | 5.80 ±0.63 |

**Values presented are means (±SE, n = 80).**

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\(^{17}\) International Journal of Chemical Studies http://www.chemijournal.com
Table 3: Effect on total plant fresh weight under the influence of moisture stress at various protective applications of BRs and Su

| Treatments | M0  | M1  | M2  | M3  |
|------------|-----|-----|-----|-----|
| Su 0       | 52.14 ±0.07 | 52.27 ±0.07 | 52.54 ±0.07 | 52.77 ±0.07 |
| Su 1       | 52.43 ±0.07 | 52.30 ±0.07 | 52.60 ±0.07 | 52.80 ±0.07 |
| Su 3       | 36.17 ±0.09 | 36.30 ±0.09 | 36.57 ±0.09 | 36.80 ±0.09 |
| M0         | 36.46 ±0.07 | 36.80 ±0.07 | 36.90 ±0.07 | 36.90 ±0.07 |
| M1         | 29.81 ±0.10 | 29.94 ±0.11 | 30.21 ±0.11 | 30.44 ±0.10 |
| M2         | 30.44 ±0.10 | 30.57 ±0.10 | 30.70 ±0.10 | 30.70 ±0.10 |
| M3         | 19.46 ±0.07 | 19.59 ±0.10 | 19.86 ±0.11 | 20.09 ±0.11 |
| NS         | 19.75 ±0.10 | 19.75 ±0.10 | 19.75 ±0.10 | 19.75 ±0.10 |

Values presented as means (±SE, n = 80). Treatments concentration in ppm: BRs = (0, 5, 10, 15, 20) and Su= (0, 1, 3, 5). M0, M1, M2 and M3 = 100, 80, 60 and 40% of the field capacity.

BRs- Brassinosteroids, M- Moisture, Su- Sucrose

Fig 1: Effect on root length under the influence of moisture stress at various protective applications of BRs and Su

Fig 2: Effect on total plant fresh weight under the influence of moisture stress at various protective applications of BRs and Su

Table 4: Effect on total plant dry weight under the influence of moisture stress at various protective applications of BRs and Su

| Treatments | M0  | M1  | M2  | M3  |
|------------|-----|-----|-----|-----|
| Su 0       | 16.36 ±0.06 | 16.40 ±0.06 | 16.48 ±0.06 | 16.56 ±0.06 |
| Su 1       | 16.49 ±0.06 | 16.49 ±0.06 | 16.56 ±0.06 | 16.56 ±0.06 |
| Su 3       | 11.35 ±0.07 | 11.39 ±0.07 | 11.47 ±0.07 | 11.55 ±0.07 |
| M0         | 11.55 ±0.07 | 11.55 ±0.07 | 11.55 ±0.07 | 11.55 ±0.07 |
| M1         | 09.35 ±0.04 | 09.39 ±0.04 | 09.48 ±0.04 | 09.53 ±0.04 |
| M2         | 09.53 ±0.04 | 09.53 ±0.04 | 09.53 ±0.04 | 09.53 ±0.04 |
| M3         | 06.11 ±0.06 | 06.15 ±0.06 | 06.23 ±0.06 | 06.3 ±0.06 |
| NS         | 06.20 ±0.06 | 06.20 ±0.06 | 06.20 ±0.06 | 06.20 ±0.06 |

Values presented as means (±SE, n = 80). Treatments concentration in ppm: BRs = (0, 5, 10, 15, 20) and Su= (0, 1, 3, 5). M0, M1, M2 and M3 = 100, 80, 60 and 40% of the field capacity.

BRs- Brassinosteroids, M- Moisture, Su- Sucrose
Fig 3: Effect on total plant dry weight under the influence of moisture stress at various protective applications of BRs and Su

References
1. Baiguzz A. An enhancing effect of exogenous brassinolide on the growth and antioxidant activity in *Chlorella vulgaris* cultures under heavy metal stress. Environmental and Experimental Botany. 2010; 68:175-179.
2. Baiguzz A, Hayat S. Effects of brassinosteroids on the plant responses to stresses. Plant Physiology and Biochemistry. 2009; 47:1-8.
3. Christina M, Le Maire G, Battie-Laclau P, Nouvellon Y, Bouillet JP, Jourdane C et al. Measured and modeled interactive effects of potassium deficiency and water deficit on gross primary productivity and light-use efficiency in *Eucalyptus grandis* plantations. Global Change Biology. 2015; 21:2022-2039.
4. Grove MD, Spencer GF, Rohwededer WK, Mandava N, Worley JF, Warthen JR et al. Brassinolide, a plant promoting steroid isolated from *Brassica napus* pollen. Nature. 1979; 281:216-7. DOI: 10.1038/281216a0.
5. Hayat S, Ahmad A. Brassinosteroids: Bioactivity and Crop Productivity. Kluwer Academic Publishers, Dordrecht. Plant cell. 2003; 79:260.
6. Hirabayashi J. On the origin of elementary hexoses. Quarterly Review of Biology. 1996; 71:365–380.
7. Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R. Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. Colloids Surfaces B: Biointerfaces. 2008; 61:298-303.
8. Mahesh B, Parshavaneni B, Ramakrishna B, Rao SSR. Effect of brassinosteroids on germination and seedling growth of radish (*Raphanus sativus* L.) under PEG-6000 induced water stress. American Journal of Plant Sciences. 2013; 4:2305–13. DOI: 10.4236/ajps.2013.412285.
9. Mitchell JW, Mandava NB, Worley JE, Plimmer JR, Smith MV, Grassi W. A family of plant hormones from rape pollen. Nature. 1970; 225:1065–66. DOI: 10.1038/2251065a0.
10. Pollle A, Rennenberg H. Physiological responses of forest trees to heat and drought. Plant Biology. 2006; 8:556-571.
11. Rana A, Ahmad M. Heavy metal toxicity in legume microsymbiont system. Journal of Plant Nutrition. 2002; 25:369-386.
12. Sasse JM. Physiological actions of brassinosteroids: an update. Journal of Plant Growth Regulation. 2003; 22:276–288.
13. Sharma N. Effect of brassinosteroid [brassinolide] on morpho-physiological, biochemical attributes and antioxidant enzyme activity in tomato (*Lycopersicon esculentum* L.) under drought stress. M.Sc. thesis, Department of genetics & plant breeding institute of Agricultural Sciences, Banaras Hindu University Varanasi-221005 U.P, India, 2016.
14. Singh S, Parihar P, Singh R, Singh VP, Prasad SM. Heavy Metal Tolerance in Plants: Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics. Frontiers in Plant Science. 2015; 6(1143):1-36.
15. Ślesak I, Haldás W, Ślesak H. Influence of exogenous carbohydrates on superoxide dismutase activity in *Trifolium repens* L. explants cultured in vitro. Acta biologica cracoviensia. 2006; 48:93-98.
16. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Avery KB et al. Contribution of working group i to the fourth assessment report of the intergovernmental panel on climate change, IPCC. The physical science basis. Cambridge University Press, Cambridge, United Kingdom and New York, USA, 2007.
17. Susiluoto S, Berninger F. Interactions between morphological and physiological drought responses in *Eucalyptus microtheca*. Silva Fennica. 2007; 41(2):221–233.
18. Yusuf M, Fariduddin Q, Ahmad A. 24-Epibrassinolide modulates growth, nodulation, antioxidant system and osmolyte in tolerant and sensitive varieties of *Vigna radiate* under different levels of nickel: a shotgun approach. Plant Physiology and Biochemistry. 2012; 57:143–53. DOI: 10.1016/j.plaphy.2012.05.004.
19. Yusuf M, Fariduddin Q, Hayat S, Hasan SA, Ahmad A. Protective responses of 28- Homobrassinolide in cultivars of *Triticum aestivum* with different levels of nickel. Archives of Environmental Contamination Toxicology. 2011; 60:68–76. DOI: 10.1007/s00244-010-9535-0.
20. Zhao B, Li J. Regulation of brassinosteroid biosynthesis and inactivation. Journal of Integrative Plant Biology. 2012; 54:746–59. DOI: 10.1111/j.1744-7909.2012.01168.x.