

Response of Tomato Cultivars on Yield and Quality Attributes Applied With Two Different Modes of BR Analogues: A Comparative Study

Hasan Syed Aiman, Irfan Mohammad, and Hayat Shamsul

Abstract—*Lycopersicon esculentum* (tomato) is widely cultivated throughout the world for its edible fruits which contains citric acid, β -carotene, ascorbic acid as well as lycopene which are powerful antioxidants, helps to prevent from lung, stomach and prostate cancer and also improve skin ability to protect against harmful UV radiation. Fruits are believed to prevent cardiovascular diseases and risks of different cancers. Brassinosteroids (BR) are the steroidal plant hormone reported to enhance the fruit productivity and grain yield. Comparative response of two BR analogues (28-homobrassinolide; HBL and 24-epibrassinolide; EBL) was tested for their two different modes of application (foliar spray and root dipping) in enhancing the quality as well as yield of tomato fruits. Two native (to India) varieties of tomato K-25 and Sarvodaya were used as a test material to check the response of mode of application and superiority of BR analogues against growth and yield.

Keywords— β -carotene, brassinosteroids, fruit-yield, lycopene, mode-of-application, tomato.

I. INTRODUCTION

IT is now well understood that Brassinosteroids (BRs) are plant growth regulators that positively regulate the plant growth under normal and stress conditions [1], [2]. It is also evident that they regulate the growth metabolism through the signals of auxins to promote cell division, root hair development, xylem differentiation, pollen development, seed setting and yield output [3]. To harvest better yield and quality products it is important to know how a plant growth regulator should be applied to the plant which is maximally enhance the desirable products [4]. The site (organ to which applied) and time of application of plant growth regulator is equally important for the better response of plant. Very a little literature available concentrating the comparative response of plant growth regulator to different mode of applications, however, different analogues of phytohormones sufficiently tried to test their comparative activity [5]-[7]. The suitability of brassinosteroid products for agricultural applications was

recognized near thirty years back, which is now have been used commercially in different parts of world including USA, Cuba, Belarus, China and India. Crop plants have been studied for the better yield output and agronomic traits regulated through BRs [8]-[12], [6], [7] including quality attributes [13], [14]. Tomato (*Lycopersicon esculentum*; Mill now *Solanum lycopersicum*; Mill) is the member of family Solanaceae is amongst the important crop plant widely grown around the world for its edible berries, rich in antioxidant constituents (citric acid β -carotene, ascorbic acid and lycopene) and minerals. The rich constituents of tomato fruits are the remedy for lung, stomach and prostate cancers due to their antioxidant properties. Ophthalmic, respiratory and cardiac ailments it is also a good for skin diseases due to its antioxidant properties. Considering the valuable source of therapeutic properties the quality and quantity aspect of tomato cultivation is important aspects for the research perspective. Here Brassinosteroids have been tested as safe, eco-friendly substitute of plant growth regulators considering its increasing acceptability for the better mode and analogue in two tomato cultivars i.e. K-25 and Sarvodaya.

II. MATERIAL AND METHODS

A. Hormone preparation

BR analogues (28-homobrassinolide/24-epibrassinolide) were obtained from Godrej Agrovet Ltd., Mumbai. Stock solution (10^{-4} M) was prepared by dissolving the hormone in 0.5 ml of ethanol, in a 100 ml volumetric flask and the final volume was made up to the mark by using double distilled water (DDW). The concentration (10^{-8} M) of HBL/EBL was prepared by dilution of stock solution in distilled water.

Surfactant (Tween-20; 0.5%) was added before the foliar spray of hormone.

B. Plant material

Seeds of tomato (*Lycopersicon esculentum*, Mill.) varieties K-25 and Sarvodaya were surface sterilized with mercuric chloride (0.01%) followed by repeated washings with DDW. The seeds were sown in earthen pots to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots in replicates under similar conditions as in case of nursery pots. Nursery plants

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were transferred to earthen pots (25 x 25 cm) at 20 day stage placed in the in a net house under the natural environmental conditions. The average temperature, humidity and day/night photoperiods were $25 \pm 20^\circ\text{C}$, $65 \pm 5\%$ and 14/10 h, respectively. Experiment was conducted in simple randomized block design during winters of 2008. Twenty days old seedlings of tomato (during transplantation) were percoated with DDW or aqueous solution of HBL (10^{-8} M) or EBL (10^{-8} M) through their root for 15 min at the time of their transplantation at a rate of 20 ml per seedling. Another set of plants at the 60 day old stage, foliage was sprayed with double distilled water (DDW) or aqueous solution of HBL (10^{-8} M) or EBL (10^{-8} M). Control plants were maintained with each treatment. The nozzle of the sprayer was adjusted in such a way that it pumped out 1 ml of DDW or HBL or EBL solutions. Irrigation was done with tap water as and when required. These plants were sampled at harvest (180 DAS) to study the growth and yield characteristics.

C. Growth characteristics

Plants were removed along with soil and dipped in water to dislodge the adhering soil particles without injuring the roots. The roots were cut from plants and blotted. The blotted roots were weighed to record their fresh weight and placed in an oven (80°C for 72 h). The samples were weighed again to record their dry weight. The weight was expressed in milligrams. The length of the roots was measured by metric scale and expressed in centimetres.

D. Yield and Quality attributes

At harvest (180 DAS), nine plants (three plants from each pot) representing each treatment were randomly sampled and counted for the number of fruits per plant and weighed to assess fruit yield per plant. Lycopene and β -carotene content, in the ripe fruits, was determined by the procedure described by [15] and [16], respectively, whereas ascorbic acid content in the fruits was determined following the procedure applied by [17].

III. STATISTICAL ANALYSES

Data were statistically analyzed using analysis of variance (ANOVA) by R (ver. 3.1.0; The R Foundation for Statistical Computing, <http://www.R-project.org>). The least significant difference was calculated for the significant data at $P < 0.05$.

IV. EXPERIMENTAL RESULTS

A. Growth characteristics

Foliar application of brassinosteroids (BRs; 10^{-8} M HBL/EBL) promoted more shoot growth than that of BRs applied as root-drenching (Fig. 1a, c, e). BRs increased root growth (root length) in present experiment; however, the length of root was smaller in the treatments where BR is directly given to the roots (root dipping). The root length was recorded higher in HBL treated plants as compared to EBL.

The effect of BRs on root fresh and dry mass was in order of EBL (root dipping) > HBL (root dipping) > EBL (foliar spray) > HBL (foliar spray). The EBL responded better as compared to HBL for either of the mode of application. Among the two varieties, K-25 performed better over Sarvodaya, where HBL provided non-significant results for length, fresh mass and dry mass of shoot and EBL remained non-significant for shoot length only. K-25 improved these parameters significantly for shoot growth parameters. The per cent increase of length, fresh and dry mass of shoot for the foliar spray of 10^{-8} M EBL was 57.56%, 61.36% and 57.01% in K-25, and 49.24%, 51.94% and 50.47% in Sarvodaya, as compared to control plants.

Almost inverse to that of shoot growth parameters, root growth was promoted more by root drenching irrespective of the analogue of BRs applied (Fig. 1b, d, f). However, again EBL was better for root growth as compared to HBL, increased significantly the root length, fresh and dry mass of two varieties except for the HBL foliar spray. K-25 excelled in its responses against the two modes and analogues of BRs. The per cent increase of root growth (length, fresh and dry mass) against the root drenching of EBL 10^{-8} M was 28.14%, 63.36% and 68.59% in variety K-25 whereas, 18.61%, 59.91% and 61.72% in variety Sarvodaya, as compared to control plants.

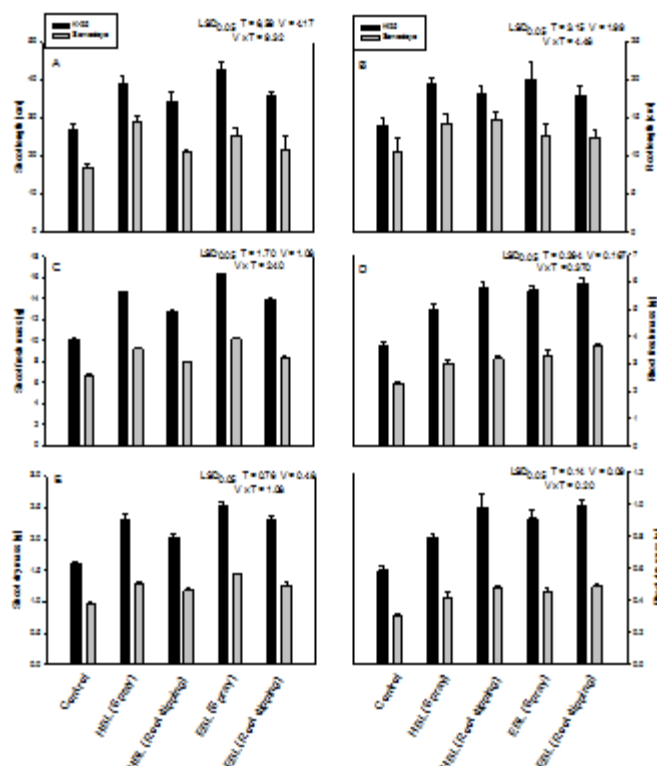


Fig. 1 Effect of two modes (Foliar spray and root dipping) of BRs (10^{-8} M; EBL and HBL) on the A) Shoot length, B) Root length, C) Shoot fresh mass, D) Shoot fresh mass, E) Root fresh mass, F) Shoot dry mass, G) Root dry mass of *Lycopersicon esculentum* var. K-25 and Sarvodaya.

B. Yield attributes

Number of fruits and fruit yield increased significantly in the tomato varieties (K-25 and Sarvodaya) received BRs (10^{-8} M HBL/EBL) through root adsorption (Fig. 2). Foliar application of BRs also increased these parameters but per cent increase was higher in plants received root application of BRs. For foliar application of HBL non-significantly increased these two parameters in Sarvodaya, whereas, K-25 showed improvement of yield attributes against all the BR applications (mode and analogues). The order of effectiveness was HBL-spray < EBL-spray < HBL-root dipping < EBL root dipping. Against the root dipping the percent increase of yield of K-25 was 49.12% (HBL) and 52.63% (EBL), whereas, in Sarvodaya it was 34.38% (HBL) and 41.67% (EBL), respectively as compared to controls.

C. Quality attributes

A non-significant difference was recorded for the quality attributes of the two varieties of tomato viz. K-25 and Sarvodaya (Fig. 2). However, BRs treatment significantly increased the lycopene and b-carotene level of tomato fruits. The responses were more encouraging for K-25 than that of Sarvodaya. Root dipping treatment proved more effective in increasing these parameters where, EBL excelled in its response. The per cent increase of lycopene content against root dipping of HBL and EBL in K-25 was 37.49% and 55.00% while in Sarvodaya it was 33.33% and 38.33%.

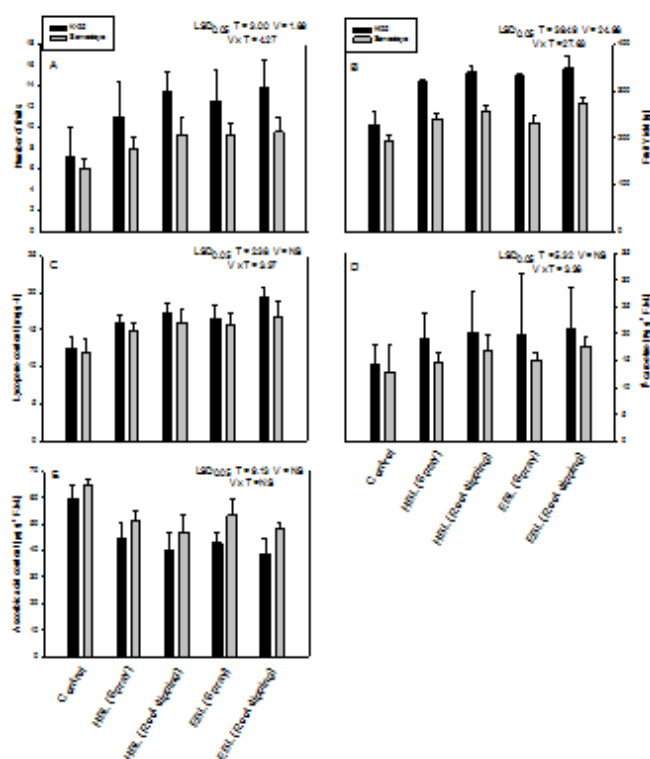


Fig. 2 Effect of two modes (Foliar spray and root dipping) of BRs (10^{-8} M; EBL and HBL) on the A) Number of fruits, B) Fruit yield, C) Lycopene content, D) β -Carotene and E) Ascorbic acid content of

Lycopersicon esculentum var. K-25 and Sarvodaya.

Likewise the increase in β -carotene content in K-25 was 41.84% and 46.27% while in Sarvodaya it was 31.17% and 36.57%, respectively, against the same treatment, compared to control plants. Contrary response was recorded for ascorbic acid content where BRs treatment decreased it in the order of HBL-spray > EBL-spray > HBL-root dipping > EBL root dipping. Maximum per cent decrease recorded against EBL (root dipping) in K-25 was 35.45% and in Sarvodaya was 24.75%, than that of control plants, in root dipping treatments.

V. DISCUSSION

With the treatment of BRs (HBL or EBL), either foliar spray or through root dipping, root and shoot growth parameters (length, fresh mass and dry mass) increased significantly (Fig. 1). However, BR application as root dipping better promoted root growth (dry and fresh mass) as compared to shoot growth. BRs contrary to other classical hormones are not transported from one place to another and thus act locally or in vicinity. It is also reported that BRs promote lateral root branching at the cost of apical root dominance [18]. The EBL improved root growth better over HBL except length where HBL performed better. However, it is also reported that BRs improve root growth inducing lateral branching at the cost of apical growth [19]. Inversely, per cent increase of shoot growth was higher in plants foliar sprayed with BRs. Although response of hormone analogue on plant is suggested to vary from plant species to species, previous findings suggests higher efficacy of EBL over HBL in most of the crop species. This seems due to structural arrangement of active groups on BR analogue rendering it more active form [9].

Brassinosteroids (BRs; HBL/EBL) improved the quality and yield attributes of tomato plants in the two selected varieties for both the modes of treatments (Fig. 2). BR mediated increased yield and quality parameters was also reported earlier by several workers [20], [10], [6], [38]. Root dipping of BRs proved more effective in improving the growth and yield of plants and quality of fruits. It is known that BRs improve the plant health improving the root hair induction and development [19], [21], [22] which enable plants to absorb minerals with root sap more efficiently. The enriched metabolic state enabled plant to flower produce more. A co-operation of BR signalling and auxins transport induced periodic pattern of shoot vasculature [23], [24] to contribute more to sink. However, foliar application of exogenous plant hormones at pre-blooming stage was reported to improve flowering and fruiting [25]. BRs has been shown to improve flowering [26] and femaleness [27] resulting in better yield attributes [2], [8], [28], [29] and reviewed by [30]. BR improves antioxidant content in legumes [14], lettuce [6], mentha [31] watermelon [32] and

tomato [13]. In this our experiment BRs significantly improved the lycopene and β -carotene level with simultaneous loss of ascorbic acid. Similar results were also reported earlier by [33] and [34] in tomato. BR regulation of source to sink relation could be the explanation better of enriched level of these pigments in tomato fruits. BR is also reported to associate with ethylene in fruit development and ripening [35], [36].

The direct application of BRs to roots of tomato plants seemingly resulted in direct assimilation of plant hormone with minimum loss at absorptive level and translocation [37]. Also there are least chances in case of BRs that they are transported from shoot to root [18] to regulate root morphology as the growth of plant increases with age. Therefore, the application of BRs at the time of transplantation is most suitable time for the application of BRs. Furthermore, EBL proved better as compared to HBL, independent of mode of application (either through root dipping or foliar application). This might be due to higher activity of EBL due to its structural arrangement of groups in steroid chain [9].

VI. CONCLUSION

Mode of application is very important aspect besides activity of phytohormones analogue used and time of application. Present experiment indicated that root absorption of BRs (EBL>HBL) proved better mode of treatment than foliar spray for nursery plant, tomato which improved the root growth and subsequent yield and quality attributes. This could be due to strengthened root system and availability of nutrients to the plant. EBL was better analogue for this application in tomato plants.

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REFERENCES

- [1] A. Bajguz and S. Hayat (2009). Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol. Biochem.* 47: 1-8. <http://dx.doi.org/10.1016/j.plaphy.2008.10.002>
- [2] Divi U. and Krishna P. (2009). Brassinosteroids confer stress tolerance. In: *Plant stress biology*: (Ed. H. Hirt). Weinheim, Wiley-VCH Verlag GmbH & Co. KGaA Pp. 119-135.
- [3] Hayat S. and Ahmad A., Fariduddin Q. (2003). Brassinosteroids: A Regulator of 21st Century. In: *Brassinosteroids*, Hayat S, Ahmad A (eds.), Springer Netherlands. Pp 231-246. <http://dx.doi.org/10.1007/978-94-017-0948-4>
- [4] Mottley J. (1983). Classification of plant growth regulators. *Jour. Agron. Educ.* 12: 64-66.
- [5] Ramírez J.A., Brosa C., Galagovsky L.R. (2005). Synthesis and bioactivity of C-29 brassinosteroid analogues with different functional groups at C-6. *Phytochemistry* 66: 581-587. <http://dx.doi.org/10.1016/j.phytochem.2004.12.029>
- [6] Serna M., Hernández F., Coll F., Amorós A. (2012). Brassinosteroid analogues effect on yield and quality parameters of field-grown lettuce (*Lactuca sativa* L.). *Scientia Hort.* 143: 29-37. <http://dx.doi.org/10.1016/j.scienta.2012.05.019>
- [7] Serna M., Hernández F., Coll F., Coll Y., Amorós A. (2013). Effects of brassinosteroid analogues on total phenols, antioxidant activity, sugars, organic acids and yield of field grown endive (*Cichorium endivia* L.). *Jour. Sci. Food Agric.* 93: 1765-1771. <http://dx.doi.org/10.1002/jsfa.5968>
- [8] Choe S., Fujioka S., Noguchi T., Takatsuto S., Yoshida S., Feldmann K. (2001) Overexpression of DWARF4 in the brassinosteroid biosynthetic pathway results in increased vegetative growth and seed yield in *Arabidopsis*. *Plant J.* 26: 573 – 582. <http://dx.doi.org/10.1046/j.1365-3113x.2001.01055.x>
- [9] Hayat S. and Ahmad A. (2003a). Brassinosteroids: Bioactivity and Crop Productivity. Springer, Netherlands. <http://dx.doi.org/10.1007/978-94-017-0948-4>
- [10] Hayat S. and Ahmad A. (2003b) Soaking seeds of *Lens culinaris* with 28-homobrassinolide increased nitrate reductase activity and grain yield in the field in India. *Ann. Appl. Biol.* 143: 121-124. <http://dx.doi.org/10.1111/j.1744-7348.2003.tb00276.x>
- [11] Pereira-Netto A.B. (2012). Brassinosteroids: Practical Applications in Agriculture, Forestry and Human Health Bentham Science Publishers, USA.
- [12] Zhang C., Ming-yi B., Chong K. (2014). Brassinosteroid-mediated regulation of agronomic traits in rice. *Plant Cell Rep.* 33: 683-696. <http://dx.doi.org/10.1007/s00299-014-1578-7>
- [13] Hayat S., Alyemeni M.N., Hasan S.A. (2012). Foliar spray of brassinosteroid enhances yield and quality of *Solanum lycopersicum* under cadmium stress. *Saudi Jour. Biol. Sci.* 19: 325-335. <http://dx.doi.org/10.1016/j.sjbs.2012.03.005>
- [14] Biesaga-Kościełniak J., Dziurka M., Ostrowska A., Mirek M., Kościełniak J., Janeczko A. (2014). Brassinosteroid improves content of antioxidants in seeds of selected leguminous plants. *AJCS* 8: 378-388.
- [15] Ranganna S. (1976). Manual of analysis of fruit and vegetable products. McGraw Hill, New Delhi. Pp. 77.
- [16] Sadasivam S. and Manickam A. (1997). Carotenes. In: *Biochemical Methods*. New Age International Publishers, New Delhi. Pp. 187–188.
- [17] Raghuramulu N., Nair M.K., Kalyanasundaram S. (1983). A manual of laboratory techniques. Natl. Instit. Nutri, Silver Prints, Hyderabad.
- [18] Symons G.M. and Reid J.B. (2004) Brassinosteroids do not undergo long-distance transport in pea. Implications for the regulation of endogenous brassinosteroid levels. *Plant Physiol.* 135: 2196-2206. <http://dx.doi.org/10.1104/pp.104.043034>
- [19] Lin L.-L., Wu C.-C., Huang H.-C., Chen H.-J., Hsieh H.-L., Juan H.-F. (2013). Identification of microRNA 395a in 24-Epibrassinolide-regulated root growth of *Arabidopsis thaliana* using microRNA arrays. *Internat. Jour.Mol. Sci.* 14: 14270-14286. <http://dx.doi.org/10.3390/ijms140714270>
- [20] Fariduddin Q., Hayat S., Ahmad A. (2003). Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in *Brassica juncea*. *Photosynthetica* 41: 281-284. <http://dx.doi.org/10.1023/B:PHOT.0000011962.05991.6c>
- [21] Mussig C., Shin G.-H., Altmann T. (2003). Brassinosteroids promote root growth in *Arabidopsis*. *Plant Physiol.* 133: 1261-1271. <http://dx.doi.org/10.1104/pp.103.028662>
- [22] Swamy K.N. and Rao S.S.R. (2010). Effect of brassinosteroids on rooting and early vegetative growth of *Coleus* [*Plectranthus forskohlii* (Willd.) Bridq.] stem cutting. *Indian J. Nat. Prod. Res.* 1: 68-73.
- [23] Mouchel C.F., Osmont K.S., Hardtke C.S. (2006). BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. *Nature* 443: 458-461. <http://dx.doi.org/10.1038/nature05130>
- [24] Ibanes M., Fabregas N., Chory J., Cano-Delgado A.I. (2009). Brassinosteroid signaling and auxin transport are required to establish the periodic pattern of *Arabidopsis* shoot vascular bundles. *PNAS*. 106: 13630–13635. <http://dx.doi.org/10.1073/pnas.0906416106>
- [25] Aliyu O.M., Adeigbe O.O., Awopetu J.A. (2011). Foliar application of the exogenous plant hormones at pre-blooming stage improves flowering and fruiting in cashew (*Anacardium occidentale* L.). *Jour. Crop Sci. Biotechnol.* 14: 143-150. <http://dx.doi.org/10.1007/s12892-010-0070-3>
- [26] Kutschera U. and Wang Z.-Y. (2012). Brassinosteroid action in flowering plants: a Darwinian perspective. *Jour. Exp. Bot.* 63: 3511-3522.

- <http://dx.doi.org/10.1093/jxb/ers065>
- [27] Papadopoulou E, Grumet R (2005). Brassinosteroid-induced femaleness in cucumber and relationship to ethylene production. *Hort. Sci.* 40: 1763-1767.
- [28] Wu C.Y., Trieu A., Radhakrishnan P., Kwok S.F., Harris S., Zhang K., Wang J., Wan J., Zhai H., Zhang C., Bai M.-Y., Chong K. (2014). Brassinosteroid-mediated regulation of agronomic traits in rice. *Plant Cell Rep.* 33: 683–696.
<http://dx.doi.org/10.1007/s00299-014-1578-7>
- [29] Jiang W.-B., Huang H.-Y., Hua Y.-W., Zhu S.-W., Wang Z.-Y., Lin W.-H. (2013). Brassinosteroid regulates seed size and shape in *Arabidopsis*. *Plant Physiol.* 162: 1965-1977.
<http://dx.doi.org/10.1104/pp.113.217703>
- [30] Vriet C., Russinova E., Reuzeau C. (2012). Boosting crop yields with plant steroids. *Plant Cell.* 24: 842-857.
<http://dx.doi.org/10.1105/tpc.111.094912>
- [31] Naeem M., Idrees M., Alam M.M., Aftab T., Khan M.M., Moinuddin (2012). Brassinosteroid-mediated enrichment in yield attributes, active constituents and essential oil production in *Mentha arvensis* L. Russ. *Agricult. Sci* 38: 106-113.
<http://dx.doi.org/10.3103/S1068367412020176>
- [32] Susila T, Amarendra Reddy S, Rajkumar M, Padmaja G, Rao PV (2012). Effects of sowing date and spraying of brassinosteroid on yield and fruit quality characters of watermelon. *World Jour. Agric. Sci.* 8: 223-228.
- [33] Vardhini V.B. and Rao S.S. (2002). Acceleration of ripening of tomato pericarp discs by brassinosteroids. *Phytochemistry* 61:843–847.
[http://dx.doi.org/10.1016/S0031-9422\(02\)00223-6](http://dx.doi.org/10.1016/S0031-9422(02)00223-6)
- [34] Ali B., Hayat S., Hasan S.A., Ahmad A. (2006). Effect of root applied 28-homobrassinolide on the performance of *Lycopersicon esculentum*. *Scientia Hort.* 110: 267-273.
<http://dx.doi.org/10.1016/j.scienta.2006.07.015>
- [35] Zarah S.S., Singh Z. (2010). Role of brassinosteroids in mango fruit ripening. *ISHS Acta Horticulturae* 934: XXVIII Inter. Hort. Cong. Sci Hort. People (IHC2010): International Symposium on Postharvest Technology in the Global Market.
- [36] Manzorra L.M., Oliveira M.G., Souza A.F., da Silva W.B., dos Santos G.M., da Silva L.R.A., da Silva M.G., Bartoli C.G., de Oliveira J.G. (2013). Involvement of brassinosteroid and ethylene in the control of mitochondrial electron transport chain in postharvest papaya fruit. *Theoret. Exp. Plant Physiol.* 25: 223-230.
- [37] Symons G.M., Ross J.J., Jager C.E., Reid J.B. (2008). Brassinosteroid transport. *Jour. Exp. Bot.* 59: 17-24.
<http://dx.doi.org/10.1093/jxb/erm098>
- [38] Takatsuto S., Matsumoto S., Fujioka S., Feldmann K.A., Pennell R.I. (2008). Brassinosteroids regulate grain filling in rice. *Plant Cell.* 20: 2130-2145.
<http://dx.doi.org/10.1105/tpc.107.055087>