



Research Article

Dissecting the Impact of Brassinolide Levels on Different Tomato Varieties

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Abstract | A field experiment was carried out to study the role of brassinolide treatments (0, 0.5 ppm, 1 ppm and 1.5 ppm) on growth and development of three tomato varieties (Yaqui, Roma and Rio Grande) at Horticulture Research Farm of Department of Horticulture, The University of Agriculture Peshawar during 2017-18. The experiment was conducted in a randomized complete block design (RCBD) with two factors replicated three times. 24-Epibrassinolide was used as a source for brassinolide. Results revealed that brassinolide and varieties significantly influenced the growth and yield parameters of tomato. Regarding different varieties, maximum number of leaves per plant (118.47), plant height (82.72 cm), number of flowers per cluster (5.91), number of flower clusters per plant (16.95) and yield (22.27 t ha⁻¹) was obtained in Rio Grande plants in contrast to Roma and Yaqui plants. Roma plants resulted in lowest blossom end rot (14.96%) as compared to Yaqui and Rio Grande. Regarding various brassinolide levels, maximum number of leaves per plant (116.41), plant height (85.48 cm), number of flowers per cluster (5.83), yield (22.24 t ha⁻¹) and decreased blossom end rot (15.31%) was noted in plants treated with 2ppm brassinolide concentration which were significantly not different from results recorded for plants applied with 1.5ppm brassinolide except for plant height. Furthermore, number of flower clusters per plant (16.75) resulted in plants treated with 1.5ppm brassinolide concentration. The interaction between varieties, brassinolide concentration and years of study revealed non-significant results for most of the studied attributes. It was concluded that treatment of tomato plants with 1.5ppm brassinolide concentration resulted in maximum growth and yield attributes.

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Keywords | Brassinolide, Plant growth regulator, Varieties, Tomato yield improvement



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Introduction

he global population is growing at a rapid pace L and is projected to reach 9.1 billion by 2050. FAO

(2023) while global average temperature is claimed to leap 1.8 to 4 °C by 2100 leading to serious concerns for food security of climate sensitive countries (Cubasch et al., 2013). Tomato being second most valuable





(Reimers and Keast, 2016) and second most widely grown crop worldwide after potato (Dorais *et al.*, 2010) encounters various biotic and abiotic stresses during its growth period. Due to its high nutritional value, demand of fresh tomato as well as tomato-based products is increasingly attracting consumer interest (Nasir *et al.*, 2015). Presently, Pakistan's tomato crop production is around 620.1 thousand tonnes ranking it 36th world wise (FAOSTAT, 2019) with an export percent in the world trade practice almost negligible.

Brassinosteroids are acknowledged as a sixth category of plant hormones and were initially reported in 1970 to have a growth promoting activity in rape (Brassica napus). Since its minute quantity isolation from 227kg of bee collected pollen, dozens of brassinosteroids from multiple plant species have been identified (Grove et al., 1979; Bajguz and Tretyn, 2003). Brassinosteroids (BRs) are found in every organ of the plant but are abundantly present in pollen and immature seeds. The quantity in pollens range from 1 to 100 ng g-1 of fresh weight while leaves and shoots have 0.01 to 0.1 ng g⁻¹ of brassinosteroids (Bajguz and Tretyn, 2003). Brassinosteroids role in enhancing plant resistance to various environmental stresses has been widely investigated through research experiments in laboratories, green houses as well as in field studies. Brassinosteroids helps the plant to pursue normal growth activities under stress condition (Clouse, 2002) and also provide protection to the plants from the harmful effects of low and high temperature stress, salinity, pathogens and injuries caused by herbicides (Khripach et al., 2000).

High temperature stress is a major constraint of tomato production in areas where the temperature goes beyond 35 degrees celsius during flower and fruit initiation. Various studies about brassinosteroids role in Arabidopsis thaliana have opened a door of advancement in brassinosteroids research which resulted in confirmation of several brassinosteroid signaling components (Clouse and Sasse, 1998; Li and Jin, 2007). Brassinosteroids have also proved its prominent role in various crops including Wheat (Sairam, 1994), Sugar beet (Schilling et al., 1991), cucumber (Pustovoitova et al., 2001) and tomato (Kamuro and Takatsuto, 1991). Tomato and brassica napus seedlings when treated with EBR resulted in enhanced tolerance to lethal heat treatment in contrast to the untreated seedlings. The brassinolide treated seedlings gathered more heat shock proteins

ultimately resulting in resistance to heat stress (Dhaubhadel et al., 1999).

Brassinosteroids have a pivotal feature of enhancing plant thermotolerance, but more systematic and thorough investigation has to be carried out on brassinosteroids functionality to modulate plant heat stress responses. To systematically explore the impact of brassinosteroids in promoting tomato growth and development, this in-depth research experiment was performed.

Materials and Methods

The experiment was carried out at Horticulture Research Farm, Department of Horticulture, The University of Agriculture Peshawar during 2017-18 with an objective to find the influence of various levels of brassinolide on selected tomato varieties. The experiment was laid out in Randomized Complete Block Design (RCBD). Two factors that are tomato varieties (Roma, Rio Grande and Yaqui) and various levels of brassinolide (0, 0.5 ppm, 1 ppm and 1.5 ppm) were studied. 24-Epibrassinolide was used as a source for brassinolide and it was provided to the plants via foliar application. Data was recorded for plant height, number of leaves per plant, number of flower clusters per plant, number of flowers per cluster, Blossom end rot (BER) (%) and yield (t ha-1). Data recorded was analyzed using Statistix 8.1 software and LSD (Least Significant Difference) test at $P \le 0.05$ was applied for mean comparison (Steel and Torrie, 1980).

Results and Discussion

Number of leaves per plant

Number of leaves per plant was significantly influenced by tomato varieties and brassinolide concentrations while the effect of year of planting and interactions were not significant. Rio Grande plants resulted in highest no. of leaves per plant (118.47), followed by (111.87) in Roma, while lowest no. of leaves per plant (103.37) were observed in Yaqui plants (Table 1). No. of leaves per plant increased significantly with the increase in brassinolide concentration. The maximum no. of leaves per plant (116.41) were recorded in plants applied with 2 ppm brassinolide which was at par with (115.76) leaves plant in plants applied with 1.5ppm brassinolide. The minimum no. of leaves per plant were noted in control plants.



Table 1: Influence of brassinolide concentrations on number of leaves plant⁻¹ of tomato varieties.

Treatment	<i>J</i>	Years	Mean
	2017	2018	
Varieties			
Roma	111.92	111.83	111.87 b
Rio Grande	118.36	118.59	118.47 a
Yaqui	103.11	103.63	103.37 c
LSD (0.05)			1.959
Brassinolide (ppm)			
0	104.62	104.09	104.36 d
0.5	107.68	108.31	107.99 c
1.0	111.42	111.92	111.67 b
1.5	115.71	115.81	115.76 a
2.0	116.23	116.60	116.41 a
LSD (0.05)			2.19
Mean	111.13	111.35	
Interactions			
YxV	NS	Y x BR	NS
V x BR	NS	$Y \times V \times BR$	NS

Means followed by similar letter(s) in column do not differ significantly from one another. NS = Non-significant and *, **= Significant at 5 and 1% level of probability, respectively.

Brassinolide considerably increased the number of leaves per in all tomato cultivars. Brassinolide is known to have a direct influence on leaves, deficiency of brassinolide or brassinolide biosynthesis blockage can lead to dark curly green leaves and hinders normal growth (Bishop and Koncz, 2002). Exogenously applied brassinolide resulted in enhanced vegetative and reproductive growth in several crops (Fariduddin et al., 2004, 2005; Bajguz and Tretyn, 2003; Hayat et al., 2001a; Ali et al., 2008; Hasan et al., 2008). Higher number and expanded leaves of tomato resulted with brassinolide application due to the influential role of brassinolide in multiple physiological processes including cell division, vascular differentiation and cell elongation (Gudesblat and Russinova, 2011). Our results are in conformity with Kang et al. (2009) who studied the influence of brassinolide treatment in cucumber plants and recorded an enhanced photosynthesis brassinolide-treated compared to control plants. An increased CO₂ uptake by soyabean plants when treated with brassinolide has also been reported which in turn increased photosynthesis (Zhang et al., 2008). Fariduddin et al. (2009) reported an enhancement in net photosynthesis with the foliar application of brassinolide combined with seed soaking. Similar positive influence of brassinolide application on net photosynthesis was also confirmed by several studies in multiple crops including geranium (Swamy and Rao, 2008) and munbean (Ali *et al.*, 2008a). Higher number of leaves per plant as well as fresh and dry weight resulted with treatment of plants with 28-homobrassinolide (Hayat *et al.*, 2001). Similarly, Irfan *et al.* (2017) also recorded an increased number of leaves, root length and plant height as a result of foliar brassinosteroid treatment of tomato plants.

Plant height (cm)

Plant height was significantly influenced by different varieties and brassinolide concentrations while the year effect and interactions were not significant. Rio Grande plants produced tallest plants (82.72 cm), followed by (77.25 cm) in Roma, while shortest plants (74.61 cm) were observed in Yaqui plants (Table 2). Concerning various brassinolide concentrations, tallest plants (85.48 cm) resulted with treatment of 2ppm brassinolide as compared to the plant height (71.86 cm) that was recorded for control plants.

Table 2: Influence of brassinolide concentrations on plant height (cm) of tomato varieties.

Treatment	Years		Mean
	2017	2018	
Varieties			
Roma	77.28	77.21	77.25 b
Rio Grande	82.80	82.64	82.72 a
Yaqui	75.51	73.71	74.61 c
LSD (0.05)			2.100
Brassinolide (ppm)			
0	72.32	71.40	71.86 e
0.5	74.76	73.92	74.34 d
1.0	77.98	77.73	77.86 c
1.5	81.51	81.33	81.42 b
2.0	86.07	84.88	85.48 a
LSD (0.05)			1.89
Mean	78.53	77.85	
Interactions			
YxV	NS	Y x BR	NS
V x BR	NS	YxVxBR	NS

Means followed by similar letter(s) in column do not differ significantly from one another. NS = Non-significant and *, ** = Significant at 5 and 1% level of probability, respectively.

Foliar application of brassinolide enhanced plant height of tomato plants in all studied varieties. There



is substantial evidence that brassinolide play a key role in plant's architecture due it's influence in regulating cell elongation and cell division. Our findings are in conformity with Sasse (2003) who investigated the role of brassinolide in various physiological processes occurring inside the plant including cell division, cell expansion, cytodifferentiation and multiple vegetative and reproductive attributes. It was concluded that exogenous brassinolide application improved various developmental pathways in plants and strengthened sinks as well as phloem uploading. Brassinolide has been reported to significantly improve root and shoot growth (Nemhauser et al., 2004) resulting in an increased plant height. The enhancement of plant height as a result of brassinolide application can also be related to efficient vascular differentiation (Ashraf et al., 2010; Caño-Delgado et al., 2004), increased cell elongation (Catterou et al., 2001) and improved chlorophyll content (Gomes, 2011) reported in various crop plants. Similar results were obtained by Irfan et al. (2017) who observed increased number of leaves, plant height, fruit number, and biomass of tomato when treated with exogenous foliar brassinosteroid application.

Tomato vegetative and reproductive growth is highly influenced by high temperature in fields during the summer season. Brassinolide treated tomato plants have been reported to accumulate more heat shock proteins as compared to non-treated plants which resulted in induced thermotolerance as well as enhanced photosynthetic efficiency (Singh and Shono, 2005). Ogweno et al. (2008) also recorded similar results with application of brassinolide in alleviating photosynthesis inhibition in tomato that resulted due to high temperature stress. Improved photosynthetic activity was observed with brassinolide treatment which protected Rubisco and other enzymes exposed to high temperature stress. Nie et al. (2017) studied the influence of overexpressing a tomato BRI1 gene on various agronomic attributes and concluded that brassinolide improved overall vegetative growth including increased plant height and lateral roots.

Number of flowers per cluster and number of flower clusters per plant

It is evident from the mean data indicated in Table 3 that varieties and brassinolide concentrations significantly influenced the number of flowers per cluster of tomato plants whereas years of plantation effect was non-significant. The interaction effects

of all the treatments were also non-significant. Regarding various varieties, Rio Grande resulted in highest number of flowers per cluster (5.91) while Yaqui plants produced the lowest number of flowers per cluster (4.78). Considering various brassinolide concentrations, an increase in number of flowers per cluster was recorded with an increase in brassinolide concentration. Highest number of flowers per cluster (5.83) observed in plants with 2ppm brassinolide concentration application which was at par with (5.80) number of flowers per cluster in plants applied with 1.5ppm brassinolide concentration. The lowest number of flowers per cluster (4.68) were produced in plants with no brassinolide application (Control).

Table 3: Influence of brassinolide concentrations on number of flowers cluster⁻¹ of tomato varieties.

Treatment	Years		Mean
	2017	2018	
Varieties			
Roma	5.11	5.13	5.12 b
Rio Grande	5.89	5.92	5.91 a
Yaqui	4.78	4.79	4.78 c
LSD (0.05)			0.147
Brassinolide (ppm)			
0	4.67	4.69	4.68 c
0.5	4.70	4.75	4.73 c
1.0	5.33	5.29	5.31 b
1.5	5.77	5.83	5.8 a
2.0	5.82	5.84	5.83 a
LSD (0.05)			0.18
Mean	5.26	5.28	
Interactions			
YxV	NS	Y x BR	NS
V x BR	NS	YxVxBR	NS

Means followed by similar letter(s) in column do not differ significantly from one another. NS = Non-significant and *, ** = Significant at 5 and 1% level of probability, respectively.

Data concerning number of flower clusters per plant is shown in Table 4. Number of flower clusters per plant of tomato plants was significantly affected by varieties, brassinolide concentrations as well as years of plantation. The interaction between varieties and brassinolide concentrations was significant while interactions of all other treatments were not significant. Rio Grande resulted in highest number of flower clusters per plant (16.95) whereas lowest number of flower clusters per plant (14.82) were recorded in Yaqui plants. Concerning various

brassinolide concentrations, the highest number of flower clusters per plant (16.75) was noted in plants treated with 1ppm brassinolide concentration while the lowest number of flower clusters per plant (15.17) resulted in control plants. Number of flower clusters per plant increased with increasing brassinolide concentration for all varieties but the highest number of flower clusters per plant produced in Rio Grande plants treated with 2ppm brassinolide concentration (Figure 1). Years of plantation has also a significant influence on number of flower clusters per plant. Highest number of flower clusters per plant (16.00) were recorded during the year 2018 as compared to (15.86) in 2017.

Table 4: Influence of brassinolide concentrations on number of flower clusters plant⁻¹ of tomato varieties.

Treatment	Years		Mean
	2017	2018	
Varieties			
Roma	15.97	16.07	16.02 b
Rio Grande	16.87	17.02	16.95 a
Yaqui	14.74	14.89	14.82 c
LSD (0.05)			0.185
Brassinolide (ppr	n)		
0	15.07	15.28	15.17 d
0.5	15.59	15.73	15.66 с
1.0	15.63	15.76	15.7 c
1.5	16.71	16.78	16.75 a
2.0	16.31	16.42	16.37 b
LSD (0.05)			0.25
Mean	15.86 b	16.00 a	
Interactions			
YxV	NS	Y x BR	NS
V x BR	*	$Y \times V \times BR$	NS

Means followed by similar letter(s) in column do not differ significantly from one another. NS = Non-significant and *, ** = Significant at 5 and 1% level of probability, respectively.

Brassinolide application significantly improved number of flowers per cluster and flower clusters plant of tomato varieties. Similar results were reported by Vardhini and Rao (2011) who carried out research experiment to explore the influence of brassinolide on growth and development of tomato under field conditions. All growth and yield related attributes including shoot length, root length, number of flowers and fruits per plant as well as fruit weight were significantly promoted with exogenous brassinolide application. Brassinosteroids are known

to have direct influence on flowering as well as fruit ripening developmental stages (Montoya *et al.*, 2005; Symons *et al.*, 2006). Pipattanawong (1996) investigated the influence of brassinolide foliar application on vegetative and reproductive attributes of strawberry plants and recorded higher number of flowers, number of flower clusters and yield per plant.

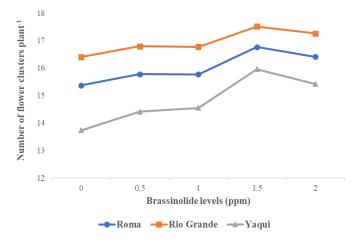


Figure 1: Interactive effect of varieties and brassinolide concentrations on number of flower clusters plant⁻¹ of tomato.

Brassinolide is known to cause an enhancement in ethylene biosynthesis in various crops including tomato (Woeste et al, 1999) and Arabidopsis (Vardhini and Rao, 2002). Papadopoulou and Grumet (2005) also reported similar results while working on the influence of exogenously applied brassinolide on cucumber plants and concluded that brassinolide significantly increased the number of flowers. These results are also in conformity with Samira et al. (2012) who resulted in higher number of flowers and fruits in pepper when applied with brassinolide exogenously. Montoya et al. (2005) studied the role of brassinolide during fruit development of tomato and noticed an increased endogenous brassinolide accumulation in early developing tomato fruits. Gomes et al. (2006) investigated the influence of foliar brassinolide application in yellow passion fruit plants and recorded an enhancement in reproductive attributes. Similar resulted were obtained by Choe et al. (2001) who recorded an improvement in various vegetative and reproductive attributes while investigating the overexpression of brassinolide biosynthetic genes in Arabidopsis. Jangid and Dwivedi (2017) studied the physiological and biochemical changes in tomato as a result of brassinosteroid treatment and nitric oxide and noticed that foliar application of both nitric oxide and brassinosteroid resulted in highest number of flower clusters per plant in tomato.



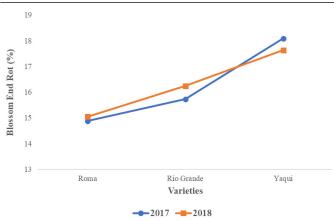


Figure 2: Interactive effect of years and varieties on blossom end rot (%) of tomato.

Blossom end rot (%)

Blossom end rot was significantly affected by different varieties and brassinolide concentrations (Table 5). Interaction between Year of planting and varieties was significant regarding blossom end rot of tomato. The year of planting effect and interactions of all other treatments were non-significant. Yaqui plants showed highest BER percentage (17.86%) while lowest percentage of BER (14.96%) was observed in Roma plants. BER incidence of tomato plants declined significantly with an increase in brassinolide level. Mean data regarding various brassinolide concentrations revealed that highest BER percentage (17.12%) was noted in control plants. The lowest BER percentage (15.31%) was observed in plants applied with 2ppm brassinolide concentration which was at par with that of (15.41%) in tomato plants treated with 1.5ppm brassinolide concentration. Interaction between year and varieties indicated that Roma plants recorded the lowest BER percentage in both the years. But the BER percentage was higher in Roma plants during the year 2018 as compared to Roma plants grown in 2017 (Figure 2).

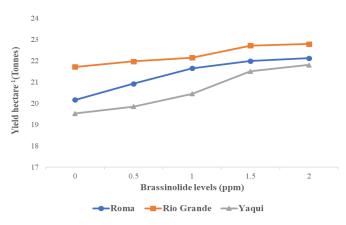


Figure 3: Interactive effect of varieties and brassinolide concentrations on yield (t ha⁻¹) of tomato.

Table 5: Influence of brassinolide concentrations on Blossom end rot (%) of tomato varieties.

ł.96 c
.96 с
.96 с
5.98 Ъ
′.86 a
235
7.12 a
5.88 ab
5.63 b
5.41 c
5.31 c
26
S
S

Means followed by similar letter(s) in column do not differ significantly from one another. NS = Non-significant and *, ** = Significant at 5 and 1% level of probability, respectively.

BER incidence was significantly reduced with treatment of tomato varieties with brassinosteroid. Low Ca²⁺ content is believed to be the cause of blossom end rot disorder in tomato. Although various other factors play a key role in regulating the blossom end rot incidence including genes involved in Ca2+ transportation, loss of xylem functionality and oxidative stress (Ikeda et al., 2017). Brassinosteroids are known for their role in enhancing cellular capacity to scavenge ROS (Liu et al., 2009) along with enabling the plants to tolerate various stress conditions (Maia et al., 2018). Brassinosteroid role in mitigating stress related issues has widely been discussed in several research works (Wu et al., 2017). Exogenously applied brassinosteroid act in plants to reduce BER incidence by regulating key enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) in stress conditions which in turn lower the Ca²⁺ disorders (Saure, 2014). Moreover, it is widely discussed in multiple research studies that Ca²⁺ deficiency leads to lipid peroxidation and reactive oxygen species generation ultimately resulting in membrane degradation. Since brassinosteroid has the ability to scavenge reactive oxygen species, its exogenous application can reduce susceptibility of





fruit to BER incidence (Turhan *et al.*, 2006). These results are also in accordance with Riboldi *et al.* (2019) who studied the role of brassinosteroid in regulating the BER incidence and recorded that brassinosteroid treatment resulted in higher soluble Ca²⁺ and antioxidant capacity which ultimately reduced the risk on BER incidence up to 44.2%.

Table 6: Influence of brassinolide concentrations on Yield $(t \ ha^{-1})$ of tomato varieties.

Treatment	Years		Mean
	2017	2018	
Varieties			
Roma	21.36	21.38	21.37 b
Rio Grande	22.26	22.27	22.27 a
Yaqui	20.65	20.60	20.62 c
LSD (0.05)			0.242
Brassinolide (pp	om)		
0	20.49	20.44	20.47 d
0.5	20.96	20.86	20.91 c
1.0	21.44	21.39	21.41 b
1.5	21.99	22.14	22.07 a
2.0	22.23	22.25	22.24 a
LSD (0.05)			0.29
Mean	21.42	21.41	
Interactions			
YxV	NS	Y x BR	NS
V x BR	*	YxVxBR	NS

Means followed by similar letter(s) in column do not differ significantly from one another. NS = Non-significant and *, ** = Significant at 5 and 1% level of probability, respectively.

Yield (t ha⁻¹)

Significant difference was observed among tomato varieties and brassinolide concentrations while years effect was not significant. Interaction between varieties and brassinolide concentration was also significant while interactions of all other treatments were not significant. Rio Grande plants recorded highest yield (22.27 t ha⁻¹), followed by (21.37 t ha⁻¹) in Roma, while lowest yield (20.62 t ha⁻¹) was obtained in Yaqui plants (Table 6). An increase in brassinolide concentration from 0.5 ppm to 2 ppm resulted in increased yield of tomato plants. Regarding various brassinolide concentrations, the highest yield (22.24 t ha⁻¹) was noted in plants applied with 2ppm brassinolide concentration which was at par with (22.07 t ha⁻¹) produced in plants treated with 1ppm brassinolide concentration. The lowest yield (20.47 t ha⁻¹) was recorded in control plants. Yield increased with increasing brassinolide concentration for all varieties, but the highest yield was noted in Rio Grande plants treated with 2ppm brassinolide concentration (Figure 2).

In the modern agricultural system, BR-derived growth promoting substances can play a great role in enhancing crop yield and yield related attributes (Wu et al., 2008). My findings indicate that brassinosteroid treatment significantly improved crop yield in all tomato varieties. This is in conformity with Hayat et al. (2012) who observed increased number of fruits and fruit yield per plant of tomato by foliar application of brassinosteroid under cadmium stress. These results might be due to the role of brassinosteroids in slowing down senescence process which resulted in higher number of fruits in brassinosteroid treated plants (Iwahori et al., 1990). Higher yield as a result of brassinosteroid application can also be related to prolonged attachment of leaves with the plant due to slower senescence resulting in higher rate of photosynthesis (Hayat et al., 2019) as well as more amount of photo assimilates transfer from source organs to sinks (Ali et al., 2006). Jangid and Dwivedi (2017) worked on the role of brassinosteroid in affecting physiological and biochemical processes of tomato and concluded an increase in Superoxide dismutase (SOD) activity, lycopene content, fruit diameter, percent fruit set, fruit yield and other yield related attributes. Irfan et al. (2017) worked on foliar application of brassinosteroid to tomato plants in order to investigate its role in growth, yield and physiological attributes of tomato.

Conclusions and Recommendations

Brassinolide application at the rate of 1.5 ppm and 2 ppm significantly enhanced number of leaves per plant, plant height, number of flowers per cluster, yield and lowered blossom end rot. Maximum number of flower clusters per plant resulted in plants treated with 1.5 ppm brassinolide concentration. Rio Grande produced highest number of leaves per plant, leaf area, plant height, number of flowers per cluster, number of flower clusters per plant and yield while Roma resulted in lowest blossom end rot percentage. Brassinolide application at the rate of 1.5ppm and variety Rio Grande is recommended for better growth and yield of tomato under the agro-climatic conditions of Peshawar.





Novelty Statement

This research study advances understanding of how brassinolide modulates plant responses and enhances yield of tomato. It provides practical recommendations for reducing blossom end rot through brassinolide application.

Author's Contribution

Gulzar Ullah: Planned the experiment, conducted the experiment, data collected and analyses, writeup of the manuscript.

Riaz Alam: Supervision, conceptualization

Gohar Ayub: Collected review of literature to support

the experiment, proof reading of the paper.

Ibrar Hussain: Facilitated in data collection, coordinated for sample collection and analyses.

Conflict of interest

The authors have declared no conflict of interest.

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