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Effects of Brassinosteroids on Growth and Biochemical Responses of Apple Plants to Water Stress

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ABSTRACT

Apple one-year-old potted "Super Chief and Red Chief" plants (Malus × domestica Borkh) were subjected to various treatments of water stress. Both varieties i.e. Super Chief and Red Chief were pre-treated by foliar application of brassinosteroid 0.05 and 0.10 ppm, three days before subjecting to stress. Growth and physio-biochemical characteristics viz. plant height, a number of branches, collar diameter, total free amino acids and drought tolerance efficiency were estimated throughout the development of water stress to showed significant reduction under water stress. The maximum reduction in Plant height was registered by plants subjected to 30 of water stress as compared to 15 days of water stress. Maximum per cent reductions in plant height i.e. 13.73 and 15.57% were observed in plants subjected to 30 days of water stress, whereas minimum reductions i.e. 4.29 and 4.91 were registered by brassinosteroid 0.05 ppm under 15 days of water stress below control in Super Chief ad Red Chief. An osmoregulating substance like total free amino acid i.e.6.04, 8.83 and 6.85, 9.45 mg g-1 showed increased accumulation with increasing duration of water stress, which helped in enhancing drought tolerance efficiency. Brassinosteroids involved in the stress response and appear to minimize the impact of stress on growth and to trigger stress resistance mechanism. However, brassinosteroid at both concentrations could help in maintaining vital growth and biochemical processes under water stress conditions. Foliar spray of brassinosteroid (0.05 ppm) prior to imposition of stress can pave the way to minimize the deleterious effects of water stress on apple plants.

Key words: *Apple, Growth, Amino acids, Brassinosteroid, Drought tolerance efficiency.*

INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) a native to South East Asia, is one of the most important and widely grown fruit crops in the temperate region of the world. Apple is the main fruit crop of farmers of hilly region of the Himachal Pradesh and cultivated in an area of 1, 11,896 hectares with a production of 4,68,134 tonnes[2]. In Himachal Pradesh, Apple is mainly growing under rainfed conditions. Most of the rainfall is received during the monsoon and winter seasons and there is very less or no rains during critical periods of growth and development[24].

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Therefore, the soil moisture generally remains very low during the hot summer months and a water stress condition develops[4]. Drought stress is one of the most adverse factors of plant growth and productivity and considered a severe threat for sustainable crop production in the conditions of changing the climate[25]. Different levels of water stress raining from normal, medium to severe, significantly affected most of the morphological, physiological, and biochemical characteristics and bud success of M9 apple and MA quince rootstocks were determined[6,23]. Plants subjected to water stress (WS) respond by a number of physiological mechanisms at the molecular, cellular, organ and whole-plant levels. These responses depend on the potency and duration of WS, as well as on the plant species and plant development stage. Drought triggers a wide variety of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Understanding the morphological, physio-biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions[1].

Brassinosteroids (BRs) are a group of naturally occurring plant steroids hormone that plays pivotal roles in plant growth and development. Foliar application of brassinosteroid on Arabidopsis thaliana and Brassica napus seedlings and expression of the stress marker gene in Br-treated and untreated seedlings role of Br in stress tolerance was observed[16]. Brassinosteroids are important for a broad spectrum of cellular and physiological processes, including stem elongation[12]. One of the most promising roles of brassinosteroid is their ability to confer resistance to a wide array of abiotic stresses. Stress protective action of brassinosteroids is the result of a complex sequence of biochemical shifts, such as activation or suppression of key enzymatic reactions, induction of protein synthesis and the production of various chemical defence compounds. The fact that brassinosteroid is natural, non-toxic and eco-friendly products, which when applied in extremely low doses are capable of improving crop yield, this makes them suitable for use in agriculture and ecology, especially under abiotic stress conditions[5].

Since water stressed plants exhibit poor growth and development, therefore, understanding of plant responses to water stress is essential for natural engineering of harder crop plants. There is lack of scientific information on effects of water stress on Red chief and Super Chief, important worldwide cultivated varieties of apple. Therefore, the aim of the study was to investigation growth, water relations and physio-biochemical changes taking place in above two varieties of apple seedlings during water stress. In addition, it was also of interest to evaluate whether brassinosteroid can help in the reversal of deleterious effects of water stress on growth and water relations in apple seedlings. To study the effects of imposed water stress and brassinosteroid on growth as well as Physio-biochemical responses of plants to imposed water stress and brassinosteroid.

MATERIAL AND METHODS

The experiment was conducted at an elevation of 1250 m above mean sea level at 30° 51'N latitude and 76° 11'E longitude in the Department of Fruit Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The experimental orchard lies under the sub-temperate, sub-humid mid-hill agro-climatic zone II of Himachal Pradesh where, summer is moderately hot during May-June while, winter is quite severe during December-January. The annual rainfall ranges between 110-120 cm and the major amount of which is received from mid-June to September. The suitable methodology has been used to understand the response of apple seedlings to different levels of imposed conditions of water stress alone and in combination with different concentrations of brassinosteroid under controlled conditions. One-year-old seedlings of apple varieties i.e. Super chief and Red chief were planted in plastic pots of 85 × 30 cm (diameter × height) and filled with soil and FYM (3:1). Planting was done under natural

condition, in the first week of February 2016. Various treatments comprised:

T_1- Control (No water stress)

T_2- Water stress for 15 days

T_3- Brassinosteroid 0.05 ppm followed by 15 days water stress

T_4- Brassinosteroid 0.10 ppm followed by 15 days water stress

T_5- Control (To compare 30 days water stress),

T_6- Water stress for 30 days

T_7- Brassinosteroid 0.05 ppm followed by 30 days water stress,

T_8- Brassinosteroid 0.10 ppm followed by 30 days of water stress

Water stress conditions were imposed by withholding watering for 15 and 30 days respectively. Before subjecting plants of both varieties to water stress conditions, pots were brought to the field capacity. Well-watered control was maintained at nearly field capacity for comparison. The plants were allowed to experience water stress conditions for 15 and 30 days withholding water. Observations regarding growth parameters, viz. plant height, collar diameter and number of branches were recorded according to standard procedures. The total free amino acid content in leaves of the same age of unstressed and stressed plants was estimated by the method described by Lee and Takahashi[17]. Drought tolerance efficiency was recorded by the method of Singh *et al*.[27]. Data on plant growth and biochemical characteristics of apple cultivars to determine the significance of differences were analyzed by using Completely Randomized Design (CRD) with four replications. All data were subjected to two way factorial ANOVA carried out using the SPSS computer package (SPSS Inc. USA).

RESULTS AND DISCUSSION

Withholding water from planted pots leads to the reduction in plant height, collar diameter and number of branch it is evident from the data presented in Table 1 to 2. All the growth characteristics *viz.* plant height, branch number and collar diameter were found higher for 30 days duration as compared to 15 days duration in both varieties *viz.* Super chief and Red chief. Among treatments, all growth parameters were maximum in control i.e. unstressed plants, whereas minimum in water stress alone. The relations between durations and treatments were found to be significant in respect of plant height. In variety Red chief maximum plant height (79.89 cm) was observed in control after 30 days of growth duration, whereas minimum plant height (64.05 cm) was recorded in plants subjected to 15 days of water stress which was statistically at par with pretreatment with brassinosteroid (0.10 ppm), followed by water stress for 15 days (64.37 cm). In variety Super chief, among 15 and 30 days of durations, number of branches was significantly higher (2.10) for 30 days duration, whereas shorter (2.01) for 15 days duration. Among the treatments, the significantly maximum number of branches (2.57) was observed in control i.e. unstressed plants. The stressed plants pre-treated with brassinosteroid (0.05 ppm) registered 2.40 branches, whereas brassinosteroid (0.10 ppm) before imposing water stress resulted in 2.16 branches. The minimum number of branches (1.20) was registered by water stress alone.

In variety Red chief, among 15 and 30 days of durations, number of branches was significantly higher (2.06) for 30 days of duration, whereas lesser (1.86) number of branches was registered for 15 days of duration. Among the treatments, the significantly maximum number of branches (2.43) was recorded in control i.e. unstressed plants. The stressed plants pretreated with brassinosteroid (0.05 ppm) resulted in 2.25 branches, whereas higher concentration i.e. 0.10 ppm before imposing water stress resulted in 2.01 branches. The minimum number of branches (1.15) was recorded in water-stressed plants. The interaction between durations and treatments were found to be non-significant in respect of number of branches.

It is evident from the data presented in Table 2 that collar diameter was influenced significantly both by different treatments as well as durations in one-year-old plants of both varieties viz. Super chief and Red chief.

In Super chief, among 15 and 30 days of durations, collar diameter was significantly higher (7.22 mm) for 30 days duration, whereas shorter (7.13 mm) in 15 days duration. Among the treatments, significantly maximum collar diameter (7.69 mm) was recorded in control i.e. unstressed, whereas minimum collar diameter (6.20 mm) was recorded in water-stressed plants. The stressed plants pre-treated with brassinosteroid 0.05 and 0.10 ppm, respectively showed 7.52 and 7.28 mm collar diameter. The interactions between durations and treatments were found significant in respect of collar diameter. In variety, Super chief maximum collar diameter (7.74 mm) was observed in control (unstressed) which was statistically at par with pretreatment with brassinosteroid 0.05 ppm followed by 30 days of water stress (7.62 mm) as well as control after 15 days of growth. Minimum collar diameter (6.13 mm) was recorded in plants subjected to 30 days of water stress. In variety Red chief, among 15 and 30 days of durations, collar diameter was significantly higher (7.18 mm) for 30 days duration, whereas shorter (6.99 mm) in 15 days duration. Among the treatments, significantly maximum collar diameter (7.56 mm) was recorded in control (unstressed), whereas minimum collar diameter (6.27 mm) was recorded in water-stressed plants. Pretreatment of stressed plants with brassinosteroid (0.05 ppm) showed 7.38 mm of collar diameter, whereas brassinosteroid, 0.10 ppm resulted in collar diameter of 7.13 mm. The interactions between water stress and treatments in variety Red chief was found to be non-significant in respect of collar diameter.

In variety Super chief and Red chief maximum free amino acid content, 57.65 and 58.51 mg g-1 respectively were recorded for 30 days of water stress. However, lesser accumulation of these substances was recorded under 15 days of unstressed plants, which were closely followed by brassinosteroid 0.05 ppm in both varieties. The interactions between durations and treatments were found significant in respect to the total free amino acid. In variety Super chief maximum free amino acid content i.e. 15.15 mg g-1 was registered by the plants subjected to 30 days of stress duration, whereas, minimum free amino acid content i.e. 4.88 mg g-1 was registered under 15 days of control i.e. unstressed plants. The stressed plants pretreated with brassinosteroid 0.05 ppm and 0.10 ppm resulted in i.e. 5.43, 7.75 and 5.97, 8.45 mg g-1 free amino acid content respectively. The interactions between durations and treatments were found significant in respect to the total free amino acid content. In variety Red chief maximum free amino acid content i.e. 16.51 mg g-1 was registered under 30 days of water stress, whereas minimum free amino acid content 4.95 mg g-1 was registered under 15 days of control i.e. unstressed plants. The stressed plants pretreated with brassinosteroid 0.05 ppm and 0.10 ppm resulted in i.e. 6.04, 8.83 and 6.85, 9.43 mg g-1 free amino acid content in that order. Drought tolerance efficiency of unstressed and stressed plants of both varieties viz. Super chief and Red chief have been depicted in Table 3. Drought tolerance efficiency was significantly influenced by pretreatment with brassinosteroid and water stress. Maximum drought tolerance efficiency i.e. 105.90 in variety Red chief was registered by brassinosteroid 0.01 ppm under 15 days of water stress, whereas minimum i.e. 95.33 was registered under 30 days of control. In variety Red chief maximum drought tolerance efficiency i.e. 115.15 was registered by brassinosteroid 0.10 ppm under 30 days of water stress in Super chief and Red chief respectively. The interactions between water stress and treatments in variety Super chief was found to be non-significant in respect of tolerance efficiency. The relations between durations and treatments were found to be significant in respect of tolerance efficiency. In variety Red chief maximum drought tolerance efficiency i.e. 115.15 was recorded in plants pretreated with brassinosteroid 0.10 ppm under 30 days of water stress, whereas minimum in 98.45 under 15 days of control.

Water stress results in the serious reduction in various crop plants including apple plants. Due to increasing worldwide shortages of water, irrigation scheduling based on sensing the drought stress directly in plants is becoming more important. Therefore, to the management of water stress and to reduce its deleterious effects on crop is important. In the present investigation the growth parameters like plant height, branch number, collar diameter were significantly influenced both by treatments as well as durations in one-year-old apple plants varieties viz. Super chief and Red chief. In both cultivars significantly maximum plant height, branch number, collar diameter was recorded in control i.e. unstressed plants which, however, reduced in water-stressed plants (Table1-3). Higher per cent reduction in growth was recorded under 30 days of water stress as compared to 15 days of stress. These findings are in conformity with those of Misger and Kumar[20] who also reported that various vegetative growth parameters like stem girth and annual shoot extension area were significantly higher in apple plants grown under higher soil moisture level as compared to those grown under restricted soil moisture levels in Red Delicious cultivar grafted on M9 rootstock.

Brassinosteroids (BRs) are plant hormones ubiquitously distributed throughout the plant kingdom and play an essential role in modulating the growth and differentiation at nanomolar to micromolar concentrations in Arabidopsis[9]. At the organism level, they promote overall growth, reproductive development, shorten the period of vegetative growth, increase crop yield and improve the quality of fruits. Brassinosteroids play a crucial role in plant development and can also promote tolerance to a range of abiotic stresses, including drought stress and temperature extremes[10].

Water deficit conditions affect the water relations in plants and the water relation characteristics are most associated with cellular growth. Lesser availability of cell water decreases the cell water potential (Ψw) which in turn induces the turgidity losses. Thus elongation rate of differentiating cells is inhibited by withholding water, cell expansion decreases along with a decrease in turgor pressure of differentiating cells resulting in smaller cell size of shoot and roots which ultimately lead to overall reduction in plant height, leaf area etc. Decreased cell size, induced by water limitation has been reported by several workers[8,14,15]. When water limitation is large enough i.e. up to 75 per cent water deficit, it leads to increased tissue water deficit and acute reduction in turgor pressure. Since cell expansion is dependent upon cell pressure potential (Ψp), developing cells will expand less and cell size will remain smaller under these conditions[7].

Our studies indicated that 0.05 ppm brassinosteroid was more beneficial in amelioration of water stress. The ameliorating capability of the BRs was found to be dose dependent[19]. The growth promoting effects of brassinosteroids under stress conditions might be attributed to their involvement in cell elongation and cell cycle progression[13,26]. Regulation of genes encoding xyloglucan endotransglucosylase hydrolases, expansions, glucanases, sucrose synthase and cellulose synthase or activation of the H+-ATPase activity by brassinosteroid was reported by Ashraf et al.[3]. Which can play an important role in the reversal of effects of water stress? Growth reduction under water stress was manifested in reduced content of nucleic acids. The decline in the levels of nucleic acids was found negated by the exogenous application of BRs[18]. Phytohormones regulate growth by affecting nucleic acid synthesis. The increase in the levels of nucleic acids might be due to enhanced synthesis and reduced degradation. Seedlings, treatment with 24-epibrassinolide, have been reported to exhibit the elevated activity of RNA polymerase and lowered activity of RNase and DNase[28].

Our studies revealed that total free amino acids accumulated substantially at the advent of water stress in both the varieties i.e. Super chief and Red chief (Table 3). Accumulation of free amino acid content in leaves of water deficit plants induced by water

stress plays an important role in osmoregulation. Rai *et al*.[22]. observed that survival and growth of plants in water stress conditions is the result of adaptation processes such as ion transport, compartmentation of an osmotic solute, synthesis and their accumulation which leads to the osmotic adjustment and protein turn over for cellular repair. The increased accumulation of amino acid content is valued in response to osmotic stress since the early accumulation of free amino acid content can contribute to a greater level of stress tolerance. Soluble sugars may function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor pressure. Therefore, soluble sugars have been specified as potential osmoregulators since, elevated sugar levels relative to control in stressed plants may contribute to the turgor maintenance[21]. Brassinosteroids resulted in the enhanced accumulation of osmolyte i.e. sugars, amino acids and proline. Osmolytes play a significant protective role in plant responses to water stress and resistance. Farooq *et al*.[11] also observed that the application of BRs increased the free proline levels in rice under drought stress.

Table 1: Effect of water stress and brassinosteroid on plant height and number of branches of apple varieties

Treatments	Super Chief			Red Chief			Super Chief			Red Chief		
	Plant height (cm)						Number of branches					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
T_1	80.10	89.93	**85.02**	69.95	79.89	**74.92**	2.52	2.62	**2.57**	2.37	2.49	**2.43**
T_2	74.12	77.59	**75.90**	64.05	67.44	**65.75**	1.15	1.25	**1.20**	1.00	1.30	**1.1 5**
T_3	76.67	79.90	**78.28**	66.51	69.75	**68.13**	2.31	2.50	**2.40**	2.1 6	2.35	**2.25**
T_4	74.52	78.04	**76.28**	64.37	67.89	**66.13**	2.07	2.25	**2.16**	1.92	2.10	**2.01**
Mean	**76.37**	**81.37**	**78.87**	**66.22**	**71.24**	**68.73**	**2.01**	**2.10**	**2.09**	**1.86**	**2.06**	**1.96**
$CD_{(0.05)}$ D	0.63			0.63			0.08			0.09		
T	0.44			0.44			0.06			0.06		
D×T	0.89			0.89			NS			NS		

Table 2: Effect of water stress and brassinosteroid on collar diameter of apple varieties

Treatments	Super Chief			Red Chief		
	Collar diameter (mm)					
	Durations					
	15 days	30 days	Mean	15 days	30 days	Mean
T_1	7.64	7.74	**7.69**	7.51	7.60	**7.56**
T_2	6.27	6.13	**6.20**	6.12	6.42	**6.27**
T_3	7.43	7.62	**7.52**	7.28	7.47	**7.38**
T_4	7.19	7.37	**7.28**	7.04	7.22	**7.13**
Mean	**7.13**	**7.22**	**7.18**	**6.99**	**7.18**	**7.08**
$CD_{(0.05)}$ D	0.08			0.10		
T	0.06			0.07		
D×T	0.12			NS		

Table 3: Effect of water stress and brassinosteroid on total free amino acids and drought tolerance efficiency of apple varieties

Treatments	Super Chief			Red Chief			Super Chief			Red Chief		
	Total free amino acids (mg g^{-1})						Drought tolerance efficiency					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
T_1	4.88	4.95	**4.91**	4.95	5.05	**5.00**	100.39	95.33	**97.86**	98.45	103.40	**100.91**
T_2	8.82	15.15	**11.99**	9.37	16.51	**12.94**	100.24	95.99	**98.12**	107.35	110.59	**108.97**
T_3	5.43	7.75	**6.59**	6.04	8.83	**7.44**	102.24	99.19	**100.71**	110.32	112.70	**111.51**
T_4	5.97	8.45	**7.21**	6.85	9.43	**8.14**	105.90	101.39	**103.64**	112.06	115.15	**113.60**
Mean	**6.27**	**9.07**	**7.67**	**6.80**	**9.96**	**8.38**	**102.19**	**97.97**	**100.08**	**107.04**	**110.46**	**108.74**
$CD_{(0.05)}$ D	0.02			0.02			2.03			2.11		
T	0.02			0.01			1.44			1.49		
D×T	0.03			0.03			2.87			2.98		

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