Effects of Gibberellic Acid on Total Carbohydrate of Shoots, Vegetative Growth and Flower Production in Barberry Plants

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Research Article

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Abstract

Background: *The time of gibberellic acid application in the non-bearing year (OFF year) makes the different responses on seedless barberry plants in it (OFF year) and next year (ON year).*

Objective: *The present research was conducted to evaluate the effects of gibberellin sprays applied at different times on barberry plants.*

Methods: *The experiment was performed in three consecutive years (2016 to 2018) in Amirabad, Birjand, Iran. The treatments included: 1) Gibberellic acid (GA3 at 200 ppm), and 2) control (0 ppm), applied six times as foliar spraying on non-bearing trees, between April and September. The foliar sprays and measuring of vegetative traits were done on non-bearing trees in 2016 and 2017. Reproductive traits evaluated on bearing trees in 2017 and 2018.*

Results: *Leaf number, width and length and internode length of current barberry shoots increased significantly. GA3 application significantly decreased by flower and fruit number, TSS/TA and anthocyanin content of fruit juice. Data showed that September might be a very important time for flower induction and differentiation. GA3 application increased the carbohydrate and phenol content of leaves and shoots.*

Conclusion: *It can be concluded that spraying gibberellic acid influences all physiological and reproductive traits of barberry plants.*

Keywords: Barberry, Carbohydrate, Flowering, Gibberellic Acid.

Introduction

Iranian seedless barberry is considered a drought tolerant plant, making it suitable for dry climates and frequent water shortages [1]. Common (European) barberry (*Berberis vulgaris L.*) has many varieties, one of which is a seedless type often called *B. vulgaris var. asperma* [2, 3]. Although barberries were reported to produce a good crop almost annually [4], we showed that barberry plants are strong alternate bearers, as the alternate bearing index (ABI) ranged from 0.63–0.71 depending on orchard location and from 0.42 to 0.69 depending on plant age [5]; the disorder increases with age and becomes more evident after 20 years [6]. Gibberellic acid has been reported to influence morphological traits, flowering, fruiting and various processes in many fruit crops [7-9].

Gibberellic acid plays a major role in stimulating cell division and cell elongation [10] and in influencing growth [11]. The application of gibberellic acid (GA) stimulates shoot growth but reduces the formation of flower buds [12-15]. The experimental data confirms the widely known phenomenon of an antagonism between vegetative growth and flowering [16]. Lim et al. [17] reported that mepiquate chloride and GA3 alone or combined, increased leaf area and chlorophyll content in grape.

GA3 increase source activity and re-distribute carbohydrate, therefore, resulting in increased sink strength of developing fruit, either through increased cell division or enhanced cell size [18, 19]. The role of GA3 on bud development is in contrast to the quantitative control of flowering, for the whole tree [20, 21]. The effect of GA3 application depends on the variety, dose and application time [22-25]. The reason for this different behavior is the heterogeneity in bud sensitivity to GA3 within an inflorescence type [26] (Guardiola, 1981). Gibberellin application is thought to inhibit flower bud development during the inductive period (late May through July in stone fruit); the first experimental evidence of this action was observed in Prunus sp. by Hull and Lewis [27] and Bradley and Crane [28].

Timing of GA application is critical in that the processes of bud development can only be affected during a limited period each year. Thus, the period of floral induction and differentiation must be known for each species, or each cultivar when there is a range of maturity date. Development of flower buds for several stone fruit was reviewed by Tufts and Morrow [29]. Flowering in deciduous perennial fruit has also been extensively reviewed by Sedgley [30]. In 'Elegant Lady' peach, a reduction of flowering was observed for GA sprays of 75 and 100 mg/L–1 applied in late May, due to a reduction in the ratio of floral: vegetative buds (Glozer, Southwick and Martin, unpublished data). There was a linear reduction in flower number as GA concentration increased from 50 to 120 m/L–1 in 'Loadel' cling peach due to GA application in July [31]. The reduction in flower number was significantly affected by time of GA spray compared with concentration, hence there was variable sensitivity to GA spray at different times of application [31]. Although there are many reports on the effects of GA3 on fruit trees, there is no study published on the effects of GA3 applications on barberry plants. Thus, the present study was undertaken to:

1. determine flower induction time of seedless barberry
2. investigate the influence of GA_3 application on performance of seedless barberry trees, particularly vegetative growth, flowering and fruiting traits.

Materials and Methods

Sampling and traits

The experiment was conducted in a barberry orchard located in Amirabad, South Khorasan, Iran, during 2016 to 2018 (three consecutive growth seasons). The orchard was irrigated once a month through a furrow system. Soil samples were collected at depths of 0-30 and 30-60 cm and 60 cm next to the trunk before spray applications and data I showed in Table 1. The treatments included: 1) Gibberellic acid (GA_3) at 200 ppm, and 2) control (0 ppm), which were applied once each month between April and September as single foliar applications to non-bearing trees that were similar in age and shape.

Depth (cm)	Soil Texture	EC ($ds.m^{-1}$)	pH
0-30	Loam-Sand	15.67	8.22
30-60	Loam	16.70	8.26

Table 1: Some physical and chemical characteristics of the soil in barberry orchard.

For estimating vegetative growth, length of new shoots (current shoot (OFF status)) and leaf number on each shoot were counted during the growing season. For measuring the length and width of leaves and length of internodes, 6 branches were selected. The flower and cluster number was calculated during full bloom, and fruit number evaluated just prior to harvest time. The leaf chlorophyll and carotenoid contents were measured by methods described by Arnon [32]. Chlorophyll fluorescence was measured by Chlorophyll Fluorometer (MINI-PAM, Walz, Germany) that measured the maximal photochemical efficiency (Fv/Fm) of photosystem II (PS II) [33]. When measuring the fluorescence parameters, all of the leaves were enveloped by foil for a dark adaptation of 20 min. This measurement was performed on leaves of ON- and OFF-year shoots one month prior fruit harvest in 2018. Shoot carbohydrate contents were determined using the anthrone method described by Mc Cready et al. [34] with a spectrophotometer (SHIMADZU AA-670, Japan). From titratable acid (TA) and total soluble solids (TSS) values, the maturity index (TSS/TA ration) was calculated. Total anthocyanin contents in fruit juice were measured by the pH differential method described by Goodwin [35]. The absorbance was measured immediately at 510 and 700 nm. The total phenol content was determined by folin-cicoalteu method at a wavelength of 725 nm and data was expressed as percentage of gallic acid [36].

Experimental design and Statistical analysis:

The experiment was conducted as factorial (GA concentrations × different spraying time) based on complete randomized block design. For each treatment, three blocks and five trees in each block were separated. Data analysis performed using analysis of variance (ANOVA) to determine statistically different values, and the means were compared considering a Fisher's Protected least significant difference (FLSD) test (at $P < 0.05$) using GenStat software (Discovery Edition, Version 9.2, 2007, VSN International Ltd., UK).

Results and Discussion

The highest shoot length was observed in the control during 2017, however, no significant differences between GA3 application and the control could be observed in 2018 (Table 2). On the other hand, GA3 application increased leaf number, internode length, leaf length and width (Table 2). There are many reports showing that Gibberellic acid (GA3) stimulate the vegetative growth [13, 37-40]. Modlibowska [41] found that GA had no effect on primary shoot growth but stimulated secondary and axillary shoot growth in pears. Mostafa and Saleh [42] stated that the foliar spraying of gibberellic acid has the ability to stimulate plant growth and development and photosynthesis. In addition, Boyers et al. [43] reported that GA_3 spraying increased the vegetative growth including shoot number and length.

Treatment	Shoot length (cm)	Leaf No. per shoot	Internode length (mm)	Leaf length (mm)	Leaf width (mm)
	2017				
Control	54.19 a	32.32 b	15.48 b	28.27 b	6.01 b
GA_3	46.89 b	53.46 a	21.28 a	29.51 a	8.94 a
			2018		
Control	48.48 a	33.85 b	15.32 b	30.52 b	7.59 b
GA_3	51.20 a	74.09 a	19.53 a	32.10 a	9.84 a

The same letter within a column denote no significant difference at 5% level of probability using LSD.

Table 2: The effect of GA3 application on shoot length, leaf number, internode length, leaf length and width of seedless barberry.

The increase in leaf number was in agreement with Xin et al. [10], Parvin et al. [44] and Neetu and Kumar [45]. The increase of leaf number by applying GA3 was showed in strawberry [46], soybean [47], faba bean [48] and bell pepper [49]. Mehraj et al. [50] stated that effects of gibberellic acid on cell division and elongation may be the cause of enhanced vegetative growth. The promotion of growth in terms of increase in plant volume and number of leaves per shoot has been attributed to increasing plasticity of the cell wall followed by hydrolysis of starch to sugars, which reduces the water potential of cell, resulting in the entry of water into the cell causing elongation through these treatments. These osmotic driven responses under the influence of gibberellins might have attributed to increase in photosynthetic activity, accelerated translocation and efficiency of utilizing photosynthetic products, thus resulting in increased cell elongation and rapid cell division in the growing portion [51].

Elliott et al. [52] indicated that the gibberellin biosynthetic enzymes and GA_3 oxidase are specifically localized in young parts, actively growing buds, leaves, and upper internodes. The role of GA in the increases of both cell elongation and cell division can be the cause of internode increase in tall peas compared with those of dwarf ones. Mitosis increases markedly in the sub-apical region of the meristem of rosette long-day plants after treatment with gibberellin [53]. Luckwill [54] found that the increased growth of shoots in the GA-treated trees was due partly to an increase in leaf number and partly to an increase in mean internode length in apple. In contrary, Luckwill [54] showed that GA had no effect on the leaf number or internode length of the dominant terminal shoot in apple trees. Gibberellic acid treatment increased leaf length compared with control. Bakeer [55] showed that all tested hedge-pruning in combination with GA3 foliar spray treatments increased leaf surface area of S-700 jojoba clone as compared with the control treatment. The obtained results of GA_3 foliar spray regarding their positive effect on leaf area was in agreement with the findings of Wasan et al. [56] who mentioned that pruning alone or in combination with spraying GA_3 produced a significant increase in leaf area of fig trees.

Spraying time	Shoot length (cm)	Leaf No. per shoot	Internode length (mm)	Leaf length (mm)	Leaf width (mm)
	2017				
April	60.73 a	62.71 a	24.09 a	31.31 a	8.00 a
May	49.24 b	38.78 bc	18.12 b	28.68 bc	7.22 ab
June	43.69 b	33.91 c	17.40 b	27.77 bc	7.25 ab
July	52.64 ab	37.63 b	16.58 b	29.29 ab	7.99 ab
August	43.54 b	41.00 bc	16.35 b	26.97 c	6.55 b
September	53.39 ab	43.31 b	17.76 b	29.33 ab	7.85 ab
	2018				
April	61.78 a	62.69 a	23.75 a	33.96 a	10.12 a
May	45.25 b	44.50 c	16.60 bc	30.87 b	7.93 c
June	47.62 b	49.76 bc	14.71 c	31.58 b	9.01 b
July	47.40 b	53.64 abc	15.50 bc	31.58 b	7.92 c
August	47.89 b	51.69 abc	15.68 bc	28.87 c	8.55 bc
September	49.11b	59.06 ab	18.31 b	31.01 b	8.76 b

The same letter within a column denote no significant difference at 5% level of probability using LSD.

Table 3: The effect of GA3 spraying time on shoot length, leaf number, internode length, leaf length and width of seedless barberry.

Data showed that the number of flowers, inflorescence and fruits significantly decreased in GA3 application, compared with control (Table 4), with the exception of inflorescence number that stayed unaffected in 2017. April spraying of gibberellin (Table 5) led to the lowest flower number and fruit production that was in agreement with finding on apples by Luckwill and Silva [57], Ramierz et al. [58], Giovanaz et al. [59], and on apricots by Son [60]. However, the highest inflorescence number was observed in this application time (Table 5). Cozens and Wilkinson [61] found that flower initiation was only prevented when GA3 was applied earlier than about five weeks before growth ceased. Later application resulted in normal flower development even though shoot growth was stimulated. Southwick et al. [31] suggested that perhaps foliar application of GA3 early in the season and at the beginning of flower induction, mostly affects differentiation of bud cells and prevents the transition of buds into productive stage, but if the foliar application is postponed to the end of flower induction period, the effect of GA3 mostly appears as killing the flower buds. Vegetative growth increases with the beginning of spring. Therefore, internal GA production enhances in shoot tip. With gibberellin foliar application, the content of this hormone multiplies in the shoot tip. It causes the shoot tip burn and the loss of the flowering positions. Regarding to data presented here, it might be found that September is critical for flower bud induction.

Treatment	Flower No.	Inflorescence No.	Fruit No.	TSS/TA	Anthocyanin (mg. L^{-1})	Fv/Fm
	2017					
Control	29.56a	17.41a	28.17a	-	-	-
GA_3	24.35b	15.73a	19.47b	-	-	-
	2018					
Control	23.00 a	19.33 a	20.54 a	74.40a	322.45a	0.13b
GA_3	21.74 b	12.88 b	13.09 b	33.60b	173.83b	0.23a

The same letter within a column denote no significant difference at 5% level of probability using LSD.

Table 4: The effect of GA_3 application on flower, inflorescence, fruit number, TSS/TA and Fv/Fm of seedless barberry.

Spraying time	Flower No.	Inflorescence No.	Fruit No.	TSS/TA	Anthocyanin (mg. L^{-1})	Fv/Fm
		2017				
April	21.49c	24.38a	15.47d	-	-	-
May	27.56b	25.01a	17.95c	-	-	-
June	26.18b	25.29a	20.83b	-	-	-
		2018				
April	19.53 c	18.26 a	16.34 c	58.40a	283.19b	0.136d
May	23.17 a	18.18 a	17.23 bc	47.90b	217.99e	0.198ab
June	23.62 a	16.58 b	16.68 c	57.90a	254.34c	0.189b
July	22.86 a	12.28 d	17.92 b	50.60b	225.12d	0.210a
August	24.03 a	16.59 b	19.66 a	47.10b	301.29a	0.165c
September	20.98 b	14.75 c	13.07 d	62.00a	206.93f	0.211a

The same letter within a column denote no significant difference at 5% level of probability using LSD.

Table 5: The effect of GA3 spraying time on flower, inflorescence and fruit number of seedless barberry

The application of GA_3 reduced significantly average number of seedless barberry fruit compared with control. The lowest levels of fruit number were seen on September 2017 and the highest fruit number obtained on August 2018 spraying (Table 5). Mostafa and Saleh [42] stated that application of GA_3 in spring was shown to be very effective in reduction of both initial and final fruit set in both spur buds and mixed lateral buds developed on one-year old shoots of Anna apple trees. On the other hand, Luckwill [62] reported that there is a strong competition between the developing fruitlets and rapidly growing shoot tips, and excessive shoot growth resulted in sparse cropping [63]. Kaur et al. [64] concluded that GA3 application (100 ppm) in May increased Sapota fruit drop (53.73%). In contrast, Ashour et al. [65] showed that foliar spray with the mixture of 100 ppm GA_3 + 100 ppm BAP + 250 ppm Boric acid, produced the highest fruit set percentage (85 and 83%). On the other hand, Abdolali and Gholamreza [66] mentioned that the application of GA_3, BAP or mixture of growth regulators did not affect fruit set percentage.

The TSS/TA ration and anthocyanin content of fruits significantly decreased under gibberellin treatment (Table 4). The TSS/TA ration showed upward and downward manner during different spraying times and the highest rate was observed in April, June and September (Table 5). The highest and the lowest anthocyanin accumulation was obtained in August and September spraying, respectively (Table 5).

Application of GA_3 significantly increased the maximum quantum efficiency (Fv/Fm) (Table 4) that was in agreement with Khandaker et al. [67]. According to Table 5, it can be seen the highest Fv/Fm was achieved in July and September and the lowest amount observed for the April application. The interaction of treatment × bearing status (ON or OFF) on Fv/Fm showed the lowest rate of this parameter in control plants. Spraying GA_3 on ON trees increased the Fv/Fm compared with OFF status (Table 8). GA_3 treatment decreased total chlorophyll content during 2017, however significantly increased this parameter during 2018 that was in agreement with Moneruzzaman et al. [68] and Zang et al. [69]. Arteca [70] suggested that application of GA increases the chlorophyll concentrations in leaves by increasing the numbers and sizes of chloroplasts and enhances the ultra-structural morphogenesis of plastids. The higher chlorophyll content may be due to greater synthesis and translocation of assimilates and water by cytokinins (CPPU) and gibberellins, which checks the degradation of chlorophyll in leaves [71]. It may be due to some kind of anti-senescence property of these growth regulators [72]. Naidu and Swamy [73] stated that application of GA_3 showed an increase in the chlorophyll content, protein content, RuBP carboxylase activity and the rate of photosynthesis that is explained by the increase rate of cyclic and non-cyclic photophosphorylation and from enhanced RuBP carboxylase activity and chlorophyll content in the leaves. In general, photosynthetic efficiency increases along with the chlorophyll concentration. GA3 has structural role in membrane of chloroplast and causes to stimulate photosynthesis [74].

Moreover, leaf carotenoid content and shoot carbohydrate accumulation stayed unaffected with this treatment during 2017, but, significantly increased with gibberellin spraying during 2018 (Table 6). The gibberellin treatment increased the carbohydrate content of leaf, leaf and shoot phenols during both years that was in agreement with Shayal Alalam [75] and Hassan et al. [76]. The time of gibberellin application also influenced total chlorophyll, carotenoid, leaf and shoot carbohydrate and phenol contents (Table 7). The highest total Chl obtained in May and September times of 2017 and 2018, respectively. The highest carotenoid content observed in September spraying time in both years. Leaf carbohydrate content increased with May and June spraying during 2017 and with September application during 2018. The highest shoot carbohydrate indicated in August application (Table 7). The highest leaf and shoot phenols were obtained in May spraying during 2017 and in April treatment during 2018 (Table 7). According to Davies [77], the GA_3 mode of action is influencing the hydrolytic enzymes related to starch, fructan, and sucrose. The increase in leaf carbohydrate content may be due to the role of gibberellic acid raising the total chlorophyll [78] that improves the process of photosynthesis [79]. Exogenous applications of GA also influence the source-sink relationship, including source and sink strengths during carbohydrate assimilation and partitioning. GA increases source strength by improving photosynthetic efficiency and improves sink strength by redistributing the photosynthetic assimilates [18, 80, 81]. Chlorophyll plays a pivotal role in photosynthetic efficiency. In general, photosynthetic efficiency increases along with the chlorophyll concentration. Thus, exogenous GA indirectly causes the Pn to increase owing to the increase in chlorophyll [82] that results in the accumulation of more dry mass [83].

Treatment	Total Chl	Carotenoid	Leaf Carbohydrate	Shoot Carbohydrate	Leaf phenols	Shoot phenols
	(mg. g-1 Leaf F. W.)		(mg. g^{-1} F. W.)		(mg Galic $acid^{-1}$ D.M.)	
2017						
Control	18.62a	1.99a	19.22b	14.61a	63.87b	64.17b
GA_3	14.82b	2.35a	23.49a	12.41a	64.51a	64.57a
2018						
Control	8.28b	1.19b	12.92b	5.47b	65.63b	64.12b
GA_3	13.05a	2.51a	14.05a	7.35a	66.29a	64.43a

The same letter within a column denote no significant difference at 5% level of probability using LSD.

Table 6: The effect of GA3 application on total chlorophyll, carotenoid, leaf carbohydrate and shoot carbohydrate of seedless barberry

Spraying time	Total Chl	Carotenoid	Leaf carbohydrate	Shoot carbohydrate	Leaf phenols	Shoot phenols
	(mg. g^{-1} Leaf F. W.)		(mg. g^{-1} F. W.)		(mg Galic $acid^{-1}$ D.M.)	
2017						
April	16.07cd	1.79c	22.82b	12.86b	64.04d	64.23e
May	18.41a	2.20b	25.27a	13.35b	64.33a	64.53a
June	16.58c	2.02bc	26.27a	10.65c	64.31a	64.36c
July	15.84d	1.79c	20.62c	13.88b	64.18b	64.39b
August	16.17cd	1.99bc	12.44d	17.69a	64.13c	64.32d
September	17.25b	3.23a	20.69c	12.67b	64.13c	64.38b
2018						
April	7.30e	1.06c	13.66b	6.86b	66.40a	64.55a
May	10.19b	1.68b	13.11b	5.84cd	66.02b	64.40b
June	12.49b	2.69a	12.84b	5.58cd	65.93c	64.16d
July	9.65c	1.56b	13.89ab	5.98c	65.46d	64.22c
August	8.67d	1.53b	12.54b	8.87a	65.95c	64.13e
September	15.69a	2.56a	15.25a	5.32d	66.02b	64.21c

The same letter within a column denote no significant difference at 5% level of probability using LSD.

Table 7: The effect of GA3 spraying time on total chlorophyll, carotenoid, leaf carbohydrate and shoot carbohydrate of seedless barberry.

Treatment	Bearing	Fv/Fm	Total Chl	Carotenoid	Leaf carbohydrate	Shoot carbohydrate
			(mg. g^{-1} Leaf F. W.)		(mg. g^{-1} F. W.)	
		2017				
Control	ON	-	14.68b	-	32.36b	11.27c
	OFF	-	13.94b	-	35.20a	13.66b
GA_3	ON	-	22.38a	-	29.88c	12.73b
	OFF	-	20.91a	-	34.91a	16.29a
		2018				
Control	ON	0.145c	21.63a	2.56a	18.38c	2.73d
	OFF	0.132c	8.93c	1.79b	25.59a	7.48b
GA_3	ON	0.267a	18.52b	1.46b	10.15d	3.63c
	OFF	0.195b	19.52b	1.81b	23.53b	11.55a

The same letter within a column denote no significant difference at 5% level of probability using LSD.

Table 8: The interaction between GA3 and bearing status of shoots on Fv/Fm, total chlorophyll, carotenoid, leaf carbohydrate and shoot carbohydrate of seedless barberry

Conclusion

Results showed that GA3 had significant effect on current shoots of barberry. Gibberellin increased leaf number, width and length and internode length in current shoots of barberry. Flower and fruit number, TSS/TA and anthocyanin content of fruit juice significantly decreased by GA3 application. Regarding to flower and fruit number, September might be very important time for flower induction and differentiation. The carbohydrate and phenol content of leaf and shoot also significantly increased by spraying gibberellin. Time of application of gibberellin also showed significant influence on all traits of this plant. It can be concluded that spraying gibberellin on different times forces significant influence on physiology, vegetative and reproductive growth and development of barberry plants.

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