Effect of 6-Benzylaminopurine Foliar Spray on *Hordeum vulgare* L. Growing in Zinc Deficient Soils

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Abstract

Hordeum vulgare is an important crop of the District, Charsadda, and the natives grow the crop mainly for food and fodder purposes. The aim of the study was to observe the effect of 6-benzylaminopurine foliar spray on the two varieties i.e., local and hybrid of Hordeum vulgare growing in Zinc deficient soils. Overall growth and phytochemical composition of the two varieties were compared during the study. The soil was analyzed for the metal presence at two different depths which is found to be high at site 10 as 4.15 mg kg^{-1} *in surface soil while in sub surface soil the maximum value was recorded as 3.90 mg kg*$^{-1.}$ *The data revealed that the Zn stress brought immense retardation in overall growth and bring decrease in chlorophyll production the proline content however produced in large amount as it is plants response toward stress. When the plants were supplied with 6-benzylaminopurine foliar spray it significantly decreases in negative impacts of Zn stress and shows decrease in proline production, increase in chlorophyll production, and increase in overall growth.*

Keywords**:** 6-Benzylaminopurine; Hordeum vulgare; Growth Parameters; Proline; Zinc.

Introduction

Any metallic entity with high density and harmful or lethal even if it is present at low quantity are termed as heavy metals (Duruibe et al., 2007). Heavy metals can be classified into three groups i.e., transition metals, basic metals, and metalloids (He et al., 2015). Toxic heavy metal reported from plants are mainly transition metals, while some are metalloids and light metals. Toxicity of heavy metals depend upon metal oxidation state and their occurrence level. From biological heavy are classified as essential metals such as Calcium, Cobalt, Copper, Potassium, Iron, Magnesium, Sodium, Manganese, and Zinc are beneficial at optimum concentration while toxic at higher concentration. Heavy metal like Silver, Aluminium, Gold, Cadmium, Mercury, Lead, Tin, and

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Thallium, they are toxic even at low quantity. From the last twenty years, heavy metals have got valuable attention. Heavy metals are referred as assemblage of metals and metalloids that contaminate the soil and bring toxicity (Duffus, 2001). Metals are found naturally in the earth crust, their level within an ecosystem varies due to different regions and spatial variations. Environmental factors and properties of the metals governed the dispersal of these metals in the environment (Khlifi & Chaffai, 2010).

A minute amount of some heavy metals like zinc (Zn) and copper (Cu) needed by plants to perform various functions (Riesen & Feller, 2005). Heavy metals are release to the environment through extensive use of pesticides, mining, sewage water and use of fertilizers (Paksoy & Acar, 2009; Pandey & Sharma, 2002). Heavy metals are being used in almost all kinds of industries. In these industrial, processes the heavy metals are used and discharge to the environment with the effluents which is a main cause of environmental pollution. As metal have nonbiodegradable nature so they cannot be destroyed and they remain and accumulates in the environment against all odds which affect all sort of life (Kojadinovic et al., 2007; Ali et al., 2024). Phytotoxicity of heavy metal is the result of changes at cellular and molecular level. In many physiological processes due to deactivating of different enzymes, it also blocks metabolically important molecules functional groups, substitution of important elements and troublesome membrane functions. The toxic effect of heavy metal promotes the production of reactive oxygen species because it alters electron transport activities. Reactive oxygen species when increases in a cell expose the cell to oxidative tension which bring peroxidation in lipids, macromolecule damage, pull apart the membrane, leakage of ions, and DNA strand cleavage (Manivasagaperumal & Sekar, 2011; Asati & Nikhil, 2016). With the industrial development of today, many dangerous chemicals (heavy metals) have been released which results in polluting air, soil and water and make it highly toxic (Asati & Nikhil, 2016; Sabullah & Shamaan, 2015; Begum et al., 2021).

Most industries of Pakistan lack the tools or not serious to take such precautions to treat the waste material before releasing to the environment, which adversely affect the medicinal plants (Asad et al., 2018). These industries therefore discharge the waste directly into the drainage system which ultimately reach agricultural lands (Ullah et al., 2009; Malik et al., 2010). Many heavy metals like As, Hg, Al, Rb, Pb, Mg, Cu and Zn are released through constant activities from volcanoes (Amaral et al., 2006). It is also observed that soil erosion also cause pollution in water and agricultural land. Heavy wind and flooding water are also the main cause of soil erosion, during the rainfall, heavy metals bound with sediments are brought to the soil here they are mixed with agrochemicals that are applied constantly to the soil and these waters being used in irrigation makes their way to the agricultural lands these sediments bound metal can survive and cause toxicity for decades (Kaizer & Osakwe, 2010; Bibi et al., 2023). When the runoff water cause erosion, it distributes heavy metals to water bodies and these wastes of heavy metals are added into drainage systems which in the end added to nearby rivers and streams (Taiwo et al., 2011).

Zinc ranked 23rd in its abundance on earth. It has atomic number of 30 and placed in the periodic table as a transition metal. The metal Zinc is present in five isotopic forms i.e., ^{64}Zn with natural abundance of 48.63%, ^{66}Zn naturally present 27.90%, ^{67}Zn that share 4.90% of all Zinc abundance, ^{68}Zn naturally 18.75% occur in the environment and ^{70}Zn which is 0.62% of total Zinc content (Weiss et al., 2005). Zinc (Zn) is an important nutrient for plants life and perform a key role in biochemical procedures. Zn is constantly required for plants growth mechanism and developmental processes. The Zn is required in minute quantities by organisms, excessive amounts of zinc can become harmful plants and become a major growth limiting factors for plants (Mirshekali et al., 2012; Misra et al., 2005). Zinc is considered as an important pollutant of environment when its presence excessed from normal limit. Its high concentration brings chlorosis, necrosis, and damage of roots. Zn when present in excess amount can bring photosynthesis inhibition and damage to plasma membrane permeability (Al Khateeb & Al-Qwasemeh, 2014).

Materials and Methods

Soil Analysis

A total of ten samples of soil were collected from ten different sites of agricultural lands of Charsadda, KP, Pakistan in three replicates. The samples were obtained at a depth of 0-30 and 30-60 cm. The soil samples were dried and sieved at a 2 mm sieve tube to remove thick root material and left over of plants. The soil samples taken in three replicates were analyzed for Ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) extractable Zn, following the method developed by Soltanpour (1985).

Experimental Plant

Hordeum vulgare L. (barley) seed were bought from a certified dealer at Tehsil bazar Charsadda. Two varieties have been taken i.e., local and hybrid to investigate the influence of 6-benzylaminopurine foliar spray and zinc stress. Three replicates were taken for each treatment. The seeds were sterilized in 5% Clorox solution for 2 minutes and washed with

50% ethanol for the purpose of sterilization. After the sterilization seeds were washed with distilled water and sown in the pots containing the sieved and prepared soil that were labelled with each concentration and variety.

Treatment of Zinc and 6-Benzylaminopurine

Fifteen seeds of both varieties i.e., local and hybrid were sown in each pot and were subjected to grow in 150ppm, 300ppm, 450ppm and 600ppm of zinc concentrations. 6 pots three for each variety were taken for control and were watered with distilled water. The various concentrations of zinc i.e., 150ppm, 300ppm, 450ppm and 600ppm were applied to each replicate of plants in amount of 5ml withholding water supply for a period of 10 days. First treatment was given to the plants after 12 days of sowing. Second treatment was given after 10 days of first treatment and third treatment were also given to plants after 10 days of the second treatment. Plants samples were collected after 26 days of sowing to check the effect of Zn stress on plants, at vegetative stage.

6-Benzylaminopurine foliar spray was prepared by adding 1ml of 6-benzylaminopurine to 100ml of water. The prepared 6-benzylaminopurine spray was sprayed on test plants by the help of spray pressure pump. Spray was applied to the plants after the one month of sowing. Second treatment of spray was given after 10 days of the first treatment. Third treatment of spray was given to the plants after 10 days of the second treatment. For determination of effects on agronomic and biochemical analysis, 3 plants from each replicate were randomly selected and were investigated for root length, shoot length, fresh weight, dry weight, moisture content, chlorophyll content and proline content. Plant chlorophyll content was determined using the standard protocol using the following formulas:

$$\text{Chlorophyll a}\left(\frac{\text{mg}}{\text{mL}}\right) = 12.7 \times \text{A663} - 2.69 \times \text{A645}$$

$$\text{Chlorophyll b}\left(\frac{\text{mg}}{\text{mL}}\right) = 22.9 \times \text{A645} - 4.68 \times \text{A663}$$

Here A645 and A663 are the absorbances at 645nm and 663nm wavelengths respectively. The proline content of the test's plants was determined using the standard method developed by Bates et al. (1973). The proline concentration was determined using a standard curve measured in µg/g.

Statistical Analysis

The experimental data was analyzed statistically using Statistics' 8.1 and Statistical Package for the Social Sciences (SPSS 16.0). The one-way ANOVA was used to compare the results followed by LSD and Dunnett test were done to compare the mean values of the data.

Results and Discussion

Soil Analysis

Soil samples were obtained from two different depths i.e., 0-30cm surface soil and 30-60cm sub soil and were analyzed for the presence of Zn. A total of ten soil samples were collected from the agricultural lands of District Charsadda, KP at 0-30 and 30-60cm depth in three replicates for each site. Ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) extractable Zn range from 1.85mg kg^{-1} at Mera prang to 2.94mg kg^{-1} at Umarzai at depth 0-30cm while at depth 30-60cm it ranges from 1.50mg kg^{-1} at Mera prang to 2.44mg kg^{-1} at Hameed mian dhere. The results show that in the 6 sites the Zn presence were found to be high and the remaining 4 sites are found to be on the low side (Table 1). The results were compared with that of the standard values published by Soltanpour (1985). These results are agreed with previously reported by Iram et al. (2018), Asad et al. (2021), and Dilawar et al. (2021).

Table 1. AB-DTPA extractable Zn concentration at two depths of 0-30 cm and 30-60 cm

S. No.	Locality	Zn concentration at depth 0-30 cm	Zn concentration at depth 30-60 cm
1	Utmanzi	1.98^{BC}	1.77^{BCD}
2	Turangzai	1.84^{C}	1.73^{BCD}
3	Umarzai	3.25^{A}	2.94^{A}
4	Paper mill Charsadda	2.53^{ABC}	2.45^{ABC}
5	Hameed mian dhere	3.09^{AB}	2.58^{AB}
6	Qazi khell	2.08^{ABC}	1.94^{ABCD}
7	Mera prang	1.78^{C}	1.44^{CD}
8	Akhon dhere	2.00^{BC}	1.40^{CD}
9	Mohmand nari	2.05^{BC}	1.60^{BCD}
10	Zarbab ghari	1.89^{C}	1.30^{D}
	Critical value	1.725	1.725

Mean values having different superscripts in column are significantly different at $\alpha < 0.05$

Shoot Length and Root Length

The comparative study of shoot length of the two varieties. local variety and hybrid variety growing under the induced stress of zinc when treated with the 6-benzylaminopurine foliar spray, showed significant increase in shoot length of both varieties. The results agreed with Mirshekali et al. (2012).

The root length of two varieties were compared in zinc stress and after the application of 6-benzylaminopurine foliar spray. The recorded data concluded that metal has the potential to toxify the plant environment and makes it harder for a plant to survive in the toxic condition and when a growth promoter was applied to the plants it shows maximum enhancement in growth and development of roots (Table 2). These results are in line with Shahid et al. (2023), whereas the root colorization also effects by abiotic stress which directly or indirectly effect the root length (Tabassum et al., 2018).

Fresh Biomass

The fresh biomass for both varieties of test plant was compared under the influence of induced stress of Zn and foliar spray. The data clearly shows that the hybrid variety was less effected as compared to the local variety and showed less inhibition rate which in fact the reason behind that the local people prefer to grow hybrid varieties instead of local varieties. The results agreed with Ahmed et al. (2013).

Table 2. Effect of 6-benzylaminopurine foliar spray on Shoot and root length of Hordeum vulgare.

Treatments	Shoot length before Spray		Shoot length after Spray	
	Shoot local	Shoot hybrid	Shoot local	Shoot hybrid
Control	12.13±0.21	15.85±0.5	60.47±0.45	60.65±0.35
150ppm	9.46±0.24*	11.46±0.41*	49.15±0.82*	55.50±6.10*
300ppm	7.93±0.46*	10.55±0.20*	45.61±0.69*	53.46±4.70*
450ppm	6.90±0.38*	9.90±0.18*	45.44±3.24*	51.13±2.25*
600ppm	5.35±0.27*	8.15±0.53*	44.43±9.15*	49.27±9.3*
Treatments	Root length before spray		Root length before spray	
	Root Local	Root Hybrid	Root Local	Root Hybrid
Control	5.16±0.13	6.45±0.5	17.77±0.36	20.45±0.23
150ppm	3.43±0.48*	4.16±0.36*	11.83±0.14*	15.45±3.19*
300ppm	2.48±0.13*	4.63±0.2*	11.61±1.32*	14.89±1.03*
450ppm	2.05±0.30*	3.93±0.04*	10.17±0.79*	15.66±1.01*
600ppm	1.93±0.21*	3.20±0.15*	10.04±1.74*	12.01±0.58*

All values in the table are grand means ± standard deviation, * = significantly different at $\alpha < 0.05$

The fresh biomass of both the varieties was compared with each other's in the influence of 6-benzylaminopurine foliar spray (Table 3). The data indicate that the plants of hybrid variety show more reduction in toxicity of Zn after the application of 6-benzylaminopurine spray. While the local variety treated with the foliar spray after the stress of Zn shows less reduction of Zn toxicity. The results agreed with Ahmed et al. (2013) and Basit et al. (2020).

Dry Biomass

The dry biomass of the two varieties were compared which each other under the induced stress of Zn. The data revealed that the local variety was more effected due to the stress of Zn as it showed more % inhibition in respect of hybrid variety. When the plants of both varieties were supplied with 6-benzylaminopurine foliar spray after the induced Zn stress (Table 3). The data indicate that Zn toxic effects were reduced by the application of 6-benzylaminopurine foliar spray and the dry biomass of the plants measured were high after the application of 6-benzylaminopurine foliar spray.

Table 3. Effect of 6-benzylaminopurine foliar spray on Fresh and Dry biomass of two varieties of Hordeum vulgare.

	Fresh Biomass before spray		Fresh Biomass after spray	
	Local	Hybrid	Local	Hybrid
Control	0.55±0.008	0.66±0.053	8.13±0.87	9.06±0.94
150ppm	0.29±0.27*	0.38±0.003*	6.10±0.27*	6.49±0.36*
300ppm	0.19±0.12*	0.29±0.015*	5.67±0.37*	6.35±0.092*
450ppm	0.16±0.023*	0.22±0.005*	4.87±1.01*	5.84±1.83*
600ppm	0.13±0.021*	0.19±0.008*	4.23±0.028*	5.10±0.83*
Treatments	Dry Biomass before spray		Dry Biomass after spray	
	Local	Hybrid	Local	Hybrid
Control	0.27±0.012	0.33±0.020	4.12±0.08	4.46±0.11
150ppm	0.15±0.015*	0.20±0.003*	2.75±0.14*	3.20 ±0.51*
300ppm	0.10±0.009*	0.15±0.009*	2.28±0.10*	2.38±0.18*
450ppm	0.08±0.001*	0.11±0.003*	1.80±0.06*	1.88±0.14*
600ppm	0.07±0.003*	0.10±0.006*	1.34±0.07*	1.85±0.01*

All values in the table are grand means ± standard deviation, * = significantly different at $\alpha < 0.05$

Moisture Content

The moisture content of both the varieties were determined in induced stress of Zn. Both the varieties were compared with each other to observe the effect of Zn stress. As both the varieties were in induced stress so it shows great decrease in moisture content but the local variety show more decrease in moisture content as compared to the hybrid variety. When the plants were supplied with the 6-benzylaminopurine foliar spray after the application of induced stress of Zn. Both the varieties were supplied with equal amount of 6-benzylaminopurine foliar spray and compare with each other to observe the reduction in Zn toxicity (Figure 1). The data indicate that the 6-benzylaminopurine foliar spray promote the growth ratio of the plants and reduce the Zn toxicity in almost all aspects.

Germination Rate

Seed germination rate per pot under the induced stress condition were determined by comparing both varieties with their control. 15 seed per pot were grown in induced stress of Zn withholding water capacity. Table 4 Shows the germination rate and % inhibition. Both the varieties were compared with their control. The results agreed with Aydinalp & Marinova (2009).

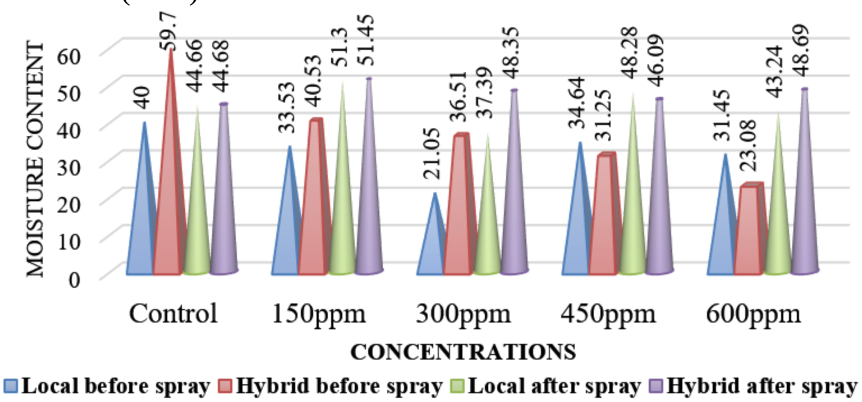

Figure 1. Effect of 6-benzylaminopurine foliar spray on moisture content of two varieties of Hordeum vulgare

Table 4. Effect of 6-benzylaminopurine foliar spray on germination rate of two varieties of Hordeum vulgare

		Hybrid variety			Local variety		
Treatments	Seeds/pot	Mean	%Ger	%Inh	Mean	%Ger	%Inh
Control	15	15	100	0	14.5	96.67	3.33
150ppm	15	12	80	20	12.5	83.33	13.79
300ppm	15	10	66.67	33.33	10.5	70	27.59
450ppm	15	7.5	50	50	8.5	56.67	41.38
600ppm	15	5.5	36.67	63.33	6.5	43.33	55.17

Chlorophyll Content

The chlorophyll A and B content were determined under induced stress of Zn for both local and hybrid varieties. The results indicate that the chlorophyll content of both the varieties shows decrease in the amount of chlorophyll content, but the local variety shows more decrease than the hybrid variety which is in fact a reason why local farmer prefer to grow hybrid variety instead of local variety because the hybrid varieties give more yield. The results agreed with Wang et al. (2009). After the determination of chlorophyll content under the induced stress the plants were supplied with the 6-benzylaminopurine foliar spray. The plant shows amazing increase in all aspects. The chlorophyll content was again measured for both the local and hybrid varieties (Table 5). After the application of 6-benzylaminopurine foliar spray the plant shows increase in all aspects. In comparison with the local variety the hybrid variety shows more reduction in stress after the application of 6-benzylaminopurine foliar spray. The results agreed with Wang et al. (2009).

Table 5. Effect of 6-benzylaminopurine foliar spray on chlorophyll a and b content of two varieties of Hordeum vulgare.

Treatment	Chlorophyll a before spray		Chlorophyll b before spray		Chlorophyll a after spray		Chlorophyll b after spray	
	Local	Hybrid	Local	Hybrid	Local	Hybrid	Local	Hybrid
Control	6.79± 0.095	6.85± 0.047	13.21 ± 0.04	14.86± 0.043	7.22± 0.021	7.37± 0.034	14.57± 0.059	15.12± 0.063
150ppm	4.6± 0.079	4.68± 0.008	10.57 ± 0.13	11.28± 0.033	5.9± 0.24	6.1± 0.036	13.24± 0.045	13.44± 0.052
300ppm	4.62± 0.059	4.63± 0.049	10.23 ± 0.07	11.13± 0.075	5.66± 0.16	5.9± 0.02	13.07± 0.044	13.14± 0.028
450ppm	4± 0.066	4.25± 0.008	10.14 ± 0.13	10.56± 0.04	4.88± 0.23	5.49± 0.05	12.57± 0.023	12.83± 0.26
600ppm	3.45± 0.08	3.87± 0.034	9.83± 0.11	10.03± 0.02	4.56± 0.25	4.76± 0.17	12.3±0.037	12.57± 0.037

Proline Content

The proline content of the both varieties were determined after the plants were treated with induce stress of Zn (Figure 2a). As proline is a defense protein which is produced more in the stress condition so when the plant was provided with more stress of 600ppm it shows more proline content of 59µ/kg local variety and 54µ/kg of hybrid variety but when we look at the condition in which the plants were provided with less stress of Zn it shows decrease in the production of proline content as plants treated with 150ppm of Zn stress local variety it shows 37µ/kg and 32µ/kg of hybrid variety. Both the control however shows littler amount of proline of 32µ/kg local variety and 30µ/kg hybrid variety. In the plants treated with the Zn stress were supplied with 6-benzylaminopurine foliar spray to observe the reduction in the Zn toxicity. After the application of 6-benzylaminopurine foliar spray the plant proline content were again measured which shows more reduction in Zn toxic effects and thus the proline content production was also reduced (Figure 2b). The data revealed that during the course of the Zn stress application both the varieties shows more production of proline but with the application of 6-benzylaminopurine foliar spray the plants shows reduction in stress and thus the production of proline also decrease as proline are stress protein that are produced in more amount during the stress condition. The results agreed with Mirshekali et al. (2012).

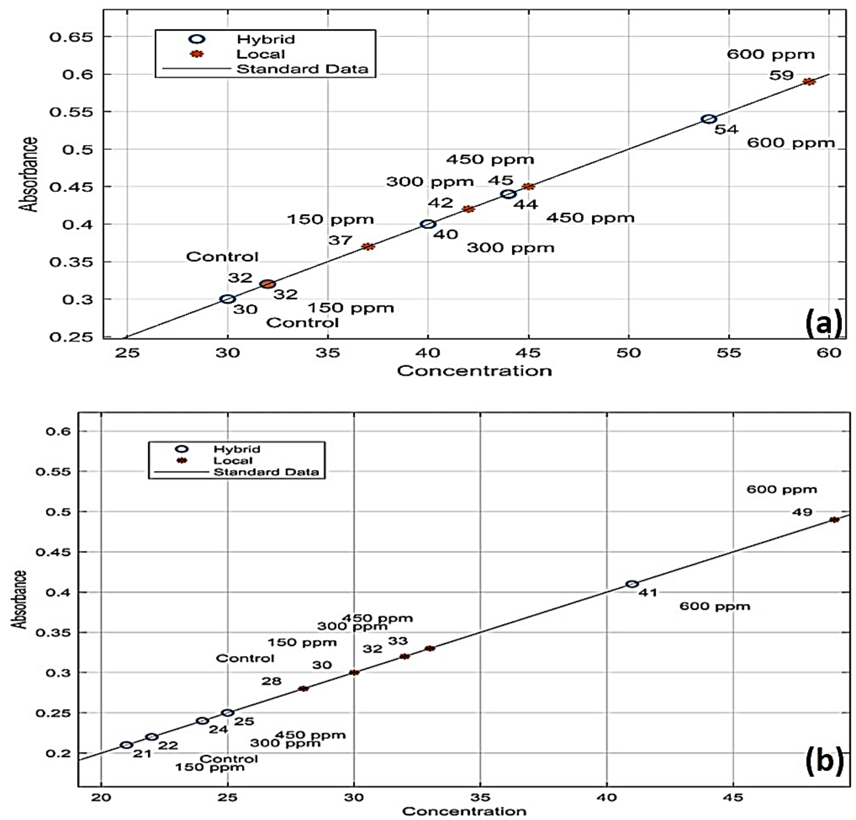

Figure 2. Effect of 6-benzylaminopurine foliar spray on Proline content of two varieties of Hordeum vulgare.

Conclusion

Hordeum vulgare L. is a major crop in the District of Charsadda, which is mostly grown by the locals for food and fodder. The metal content of the local soil was measured at two distinct depths, and the results showed that the greatest value in subsurface soil was 3.90 mg kg^{-1}, while the highest value was observed at site 10 at 4.15 mg kg^{-1}. The findings showed that the Zn stress significantly slowed down overall development and reduced the formation of chlorophyll. Proline content, on the other hand, was still generated in considerable amounts as a plant's stress response. When 6-benzylaminopurine foliar spray was applied to the plants, the detrimental effects of zinc stress were greatly reduced. Proline synthesis decreased, chlorophyll production increased, and overall growth increased.

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