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Exogenous Growth Regulators and Water Stress Enhance Long-Term Storage Quality Characteristics of Onion

Goraksha C. Wakchaure [1,*], Pratapsingh S. Khapte [1], Satish Kumar [1], Paramsivam Suresh Kumar [2], Leo Sabatino [3,*] and Pradeep Kumar [4]

[1] ICAR—National Institute of Abiotic Stress Management, Baramati 413115, Maharashtra, India
[2] ICAR—National Research Centre for Banana, Tiruchirapalli 620102, Tamil Nadu, India
[3] Department of Agricultural, Food and Forest Sciences, University of Palermo, 90128 Palermo, Italy
[4] ICAR—Central Arid Zone Research Institute, Jodhpur 342003, Rajasthan, India
* Correspondence: goraksha.wakchaure@gmail.com (G.C.W.); leo.sabatino@unipa.it (L.S.)

Abstract: Exogenous growth regulators (GRs) play a crucial role in alleviating water stress and sustaining crop yields in water-stressed areas. However, their effects on onions post-harvest quality, particularly post-monsoon onion—often preferred for long-term storage—were never studied. Therefore, this led us to investigate the interaction between water stress and GRs on the physiochemical and functional quality attributes of onions during long-term storage (9 months, at 25 ± 1 °C and 65 ± 5% RH). Onion crop was raised under four water stress levels i.e., 1.00–0.85, 0.84–0.70, 0.69–0.40, and 0.39–0.10 IW: CPE, designated as no, low, medium, and severe water stress, respectively, using a line source sprinkler system (LSS). GR treatments include potassium nitrate (PN, 15 g L^{-1}), sodium benzoate (SB, 100 mg L^{-1}), thio-urea (TU, 450 ppm), and gibberellic acid (GA, 25 ppm). Results reveal that the significant temporal changes in the dry matter, rehydration ratio, total soluble sugar (TSS), protein, and total phenolics content (TP) of the onion bulbs during storage, indicate the cumulative impact of the treatments on overall physicochemical status. Water stress increased onion biochemical attributes, especially pyruvic acid content, superoxide dismutase (SOD), and peroxidase (POD) activity. Storage quality of onions progressively decreased with the increase in storage period. Stressed onions, especially those produced under severe water stress condition, showed high weight losses, presenting poor keeping quality. However, application of GRs, especially SB, TU, and PN, reduced bulb weight losses together with maintaining slightly better bulb physicochemical properties, thereby improving the overall storage quality, particularly with a moderate level of water stress (0.69–0.40 IW:CPE). The exogenous application of GRs with moderate water stress is suggested as a key strategy in improving the keeping quality of onion bulbs and ensuring its availability during the lean season.

Keywords: onion; growth regulators (GRs); water stress; storage; physiochemical changes; pungency

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1. Introduction

India contributes to about 20% of global onion production next to China. Onion (*Allium cepa* L.) is mainly cultivated in high to extremely high water-stressed arid and semi-arid regions, both during monsoon (May–October) and post-monsoon (November-April) seasons in India. Around 50% of the latter season production is subjected to long-term storage for use in lean season (August–November); farmers also fetch good prices due to its comparatively superior quality and longer shelf life. However, the post-monsoon crop is more vulnerable to water stress caused by a limited availability of irrigation water and unfavourable weather conditions. These conditions adversely influence the bulb yield and its physical attributes, such as average bulb weight, bulb size, uniformity, and sphericity [1]. Furthermore, water stress substantially alters the chemical composition and antioxidant properties of fresh onion bulbs [2]. Pelter et al. [3] reported that the percentage of single-centered bulbs, an important quality parameter of onions to meet demand of both

processing and storage, was lowered by 40% when the soil–water stress occurred earlier in the growing season than when the stress occurred later (18%). Furthermore, unstable market price, lack of adequate storage, and market infrastructure cause instability in onion production and supply. Thus, relatively higher post-harvest storage losses (ranging from 40 to 90%) were reported in India [4]. Moreover, the quality of onion bulbs is highly vulnerable to water loss, sprouting and rooting incidences, and changes in chemical composition [5]. Therefore, adaptation of efficient storage strategies for minimizing storage losses should be prioritized, so as to maintain the quality of onion bulbs during long-term storage and ensure year-round availability of high-quality onions in the market.

Storage of onions is a multifaceted task involving several pre-harvest and post-harvest factors, such as genotypes, cultural practices, cropping environment, curing methods, and storage conditions (temperature and humidity). These factors control physiological and metabolic activity of onions, thus ultimately influencing the physicochemical and phytochemical properties until they reach the consumer [6]. Post-harvest sprouting is a major physiological factor limiting the storage period and changing the storage quality of onion [7]. During storage, onions lose quality due to the high catabolism of carbohydrates and phytochemicals. Several changes in physicochemical properties of onions during long-term storage were reported by previous researchers, e.g., degradation of dry matter content, skin integrity, intactness and colour [8], changes in flavor precursors, pungency, and sugar content [9], irregular variations in quercetin, flavonoids, pyruvic acid, enzymes (peroxidase activity) and sugar contents [6], and total phenol content and antioxidant activity [7]. All these reports highlight the problem of post-storage deterioration of onions, demanding keen attention of the scientific managers and food industrialists. In order to protect onion quality during storage, mass-growers, sellers and food industries practice various pre- and post-harvest treatments. The use of mitosis inhibitors and growth regulators, among others, are some of these methods. Plant growth regulators are found to be more effective in controlling hormonal activities (GA, auxin, and cytokinin) mainly responsible for dormancy and sprouting in stored onions. As regards onion storage, some of the GRs act interdependently, while others act independently; however, current knowledge of these relationships and their relative responses is in infancy [10].

The beneficial effects of GRs in prolonging the storage life of onions through regulation of physiological processes, such as maintaining dormancy and delaying sprouting, were elaborated through several reports. Ethrel, ethephon, herbal extracts, and maleic hydrazide (MH) were reported for their role in enhancing onion storage commercial viability [11]. Wakchaure et al. [1] reported that salicylic acid, sodium benzoate, thiourea, and potassium nitrate applied during different onion growth stages can improve growth and bulb yield under water-scarce conditions. Plant growth regulators enhance the plant's ability to cope with stress conditions through nutrient allocation, growth regulation, and source–sink transitions. However, to the best of our knowledge, their roles in changing the physiochemical and functional characteristics of onions produced in water deficit and long-term storage conditions are yet to be explored. Furthermore, there are no reports on the effects of these growth regulators on storage quality aspects of onion. Therefore, the present study aimed to investigate the interactive effect of exogenous application of selected GRs and water stress on yield and the physiochemical and functional characteristics of onion bulbs during long-term storage.

2. Materials and Methods

2.1. Treatments, Sampling, and Storage of the Onion Bulbs

Red onions (*Allium cepa* cv. Bhima Kiran) were grown at the research farm located at ICAR–National Institute of Abiotic Stress Management Baramati (Maharashtra, India) during the post-monsoon season (November 2020–April 2021). Four different water stress levels were simulated using a line source sprinkler system (LSS) with irrigation water (IW) applied equaling 1.00–0.85 (628–549 mm), 0.84–0.70 (548–471 mm), 0.69–0.40 (470–313 mm),

and 0.39–0.10 (312–156 mm) times cumulative open pan evaporation (CPE); these treatments were rated, respectively, as no, low, medium, and severe water stress (Figure 1).

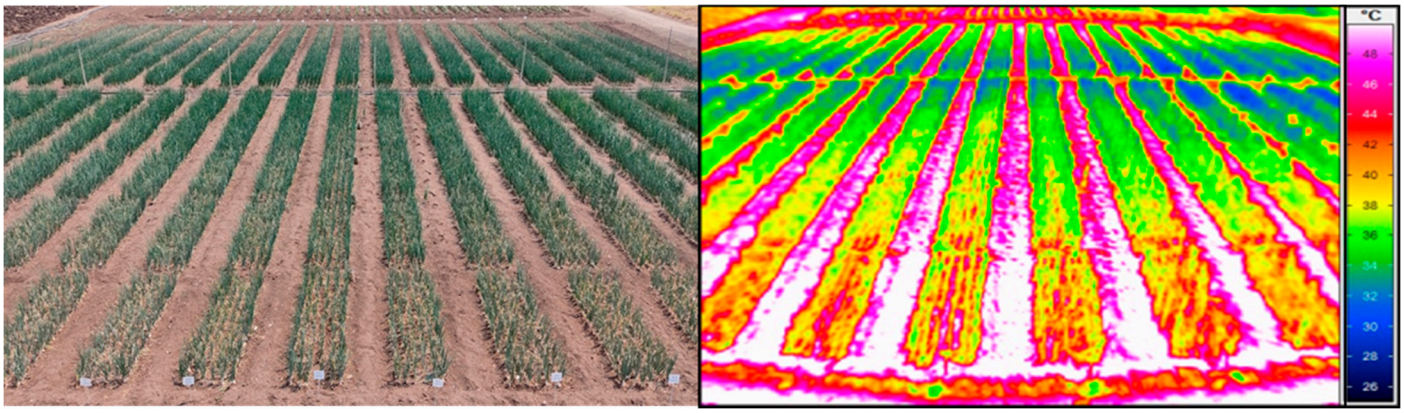

Figure 1. Visible image (**Left**) and thermal image (**Right**) of the line source sprinkler experimental set-up in the field.

The first dose of growth regulators viz., sodium benzoate (SB, 100 mg L^{-1}), potassium nitrate (PN, 15 g L^{-1}), thio-urea (TU, 450 ppm), and gibberellic acid (GA, 25 ppm) was applied just 2 weeks before harvest when enough green foliage was present. Then, the harvested onion samples were cured in the open shed for 10 days, trimmed for roots and leaves, and shifted to the laboratory. Bulbs in the weight range of 161.4–176.0, 140.5–161.0, 86.2–140.0, and 42.4–86.0 g with 68.1–73.4, 65.3–68.0, 55.1–65.2, and 46.3–55.1 mm geometric mean diameters produced under no, low, medium, and severe stress, respectively, with no visible defects were selected for the storage study (Figure 2). The second dose of the same growth regulators was applied on outer bulb surfaces at the onset of storage. Then, onion bulbs of respective treatments were filled separately in nylon wire-mesh bags (5 kg capacity) and placed in three batches comprising 20 bags each, under temperature (25 ± 1 °C) and relative humidity (65 ± 5%)-controlled chambers (Model: DR36VL, R−134A refrigerant, Percival Scientific, Perry, IA, USA). All bulbs were labelled and weighed individually in each bag to measure weight loss during storage. In all experimental batches, three bulbs were sampled randomly from each bag for analysis initially (0 days), followed by an interval of one month, until the ninth month. All adhering debris from selected bulbs was thoroughly washed away with tap water; the outer skins and ends of the cleaned bulbs were removed, and the inner material was homogeneously chopped into smaller (5 mm) pieces. To obtain a representative sample, the pieces were thoroughly mixed.

Figure 2. Onion bulbs of cv. Bhima Kiran in different levels of water stress from a left to right (no stress, low, moderate, and severe).

2.2. Measurement of Dry Matter Content and Rehydration Ratio

The dry matter (DM) content with corrected values of onion bulbs were measured using the method suggested by Sharma and Lee [6]. The DM of chopped bulb samples of 25–30 g were determined after drying first at 80 °C for 24 h and then at 105 °C for 2 h in a hot air oven. Rehydration ratio (RR) was determined according to Ranganna [12]. In brief, five grammes of dried bulb samples were added to 150 mL of distilled water, boiled for 15 min, cooled, and the RR was determined by draining off the excess water.

2.3. Estimation of Physiochemical and Functional Characteristics

2.3.1. Total Soluble Sugar (TSS)

The TSS content from the bulb tissues was determined according to Yemm and Willis [13]. Briefly, 50 mg of bulb-tissue was digested in 2.5 mL of 2.5 M HCl for 180 min. Digested samples were then cooled at ambient temperature, the acid was neutralized by adding excess sodium carbonate, and diluted to 50 mL with milli q water. Then, the samples were centrifuged at 7830 rpm/10 min, and 1 mL aliquot of the supernatant was mixed with anthrone reagent (0.2% anthrone in 95% ice-cold H_2SO_4). The mixture was kept in a boiling water bath for 8 min, cooled on ice, and observations were recorded at 630 nm using a spectrophotometer. Milli q water served as control, while the calibration curve was plotted using glucose standard. Sugar concentration from the samples was determined using standard curves and expressed in mg/g FW.

2.3.2. Protein Content

Estimation of total proteins (mg/g FW) from the tissue samples was conducted using Lowry's method. Briefly, 0.5 gm of the tissue samples were homogenized in chilled phosphate-EDTA buffer (100:1 mM; pH 7.2). Debris was pelleted by centrifugation at 10,000 rpm for 10 min, and 0.1 mL aliquot was mixed with freshly prepared reagent (mixture of 2% Na_2CO_3 in 0.1N NaOH; and 0.5% $CuSO_4 \cdot 5H_2O$ in 1% sodium potassium tartarate), mixed well and kept under ambient conditions for 10 min. The mixture was then added with 0.5 mL of Folin Coicalteau reagent, mixed well, and kept in dark at ambient conditions for 30 min. Observations were recorded at 660 nm. The calibration curve was plotted using bovine serum albumin (BSA).

2.3.3. Antioxidant Enzymes Estimation

One gram of freshly obtained tissue samples was homogenized in an ice-cold phosphate buffer (100 mM; pH 7.5; and EDTA 0.5 mM). Samples were centrifuged at 10,000 rpm for 10 min to obtain the supernatant containing crude enzyme.

Superoxide Dismutase (SOD)

The superoxide dismutase activity was analyzed through a reaction system consisting of methionine (13.33 mM); nitroblue tetrazolium chloride (NBT) (75 µM); EDTA (0.1 mM); phosphate buffer (50 mM; pH 7.8); sodium carbonate (50 mM); crude enzyme (0.1 mL); and riboflavin (2 µM). The system was illuminated under fluorescent lamps (15 W) for 15 min; followed by being kept in dark for another 15 min. Milli q water served as control. Finally, the observations were recorded at 560 nm. The enzymes activity was expressed in terms of U/mg protein. Units of enzyme were calculated in terms of 50% reduction in absorbency over the control.

Peroxidase Activity (POD)

Activity of peroxidase from the samples was estimated in terms of guaiacol oxidation. Briefly, the reaction mixture contained guaiacol (16 mM); H_2O_2 (2 mM); phosphate buffer (50 mM; pH 6.1); and crude enzyme (0.1 mL). Development of the colour was monitored at 470 nm. Enzyme activity was calculated as per Castillo et al. [14].

2.3.4. Total Phenolics (TP)

The total phenolics (TP) from the bulb tissues was quantified using the Folin–Ciocalteu method and expressed in terms of gallic acid equivalent (GAE) in mg/ g FW. Tissue samples (0.1 gm) were homogenized in 2.0 mL of ice-cold 80% methanol. Debris was removed by centrifugation at 10,000 rpm for 10 min; and 0.1 mL aliquot from the supernatant was mixed with 0.5 mL Folin Ciocalteau (FC) reagent (1.0 M) and kept for 3 min at ambient conditions. Then, the mixture was added with 2.0 mL of Na_2CO_3 (20%), mixed well, and kept in a boiling water bath for 1 min, then cooled under ambient conditions. The absorbance was recorded at 650 nm. Gallic acid served as standard, while milli q water served as control.

2.3.5. Pyruvic Acid Content

The pyruvic acid content was estimated according to Teare Ketter and Randle [15]. Fresh tissue samples (5 g) were homogenized in 4 mL of milli q water with the help of mortar and pestle; the slurry was kept undisturbed under ambient conditions for 10 min. Then, 0.5 mL of the slurry was thoroughly mixed with 1.5 mL of 5% trichloro-acetic acid (TCA) and allowed to stand for 1 h under ambient conditions. Then, the slurry was mixed with 18 mL of milli q water and used for quantitation of pyruvic acid. Quantity of pyruvic acid was expressed in terms of μmol/g FW.

2.4. Statistical Analysis

Data in storage experiments were analyzed in three replicates using SAS software (Ver. 9.3) for all determinations. A three-way ANOVA, with the factor growth regulators (GR), water stress (WS), and storage time (T) was carried out to assess the statistical significance among main and interaction effects (GR × WS, GR × T, WS × T, GR × T, GR × WS × T) for all analyzed parameters. Duncan's multiple range test was further performed to determine least significant difference (LSD) at $p = 0.05$ between means within factors.

3. Results and Discussion

In the present study, the most popular light-red onion cultivar, 'Bhima Kiran', was chosen because of its high storability and potential yield in water-scarce regions. During storage, onion bulbs pass the rest, dormancy, and sprouting phases. Thus, the physiochemical composition of onions may be expected to change under the influence of exogenous growth regulators and water stress.

3.1. Changes in Dry Matter and Weight Loss of Stored Onion

The moisture content of onion bulbs tends to reduce during prolonged storage. Similarly, dry matter (DM) content was also reported to decline over time. This in turn negatively influence bulb quality, and hence produce marketability [16]. Therefore, improved DM content in onion bulbs under long-term storage represents a major challenge towards maintaining adequate bulb quality. In the study, DM content differed significantly due to GRs and water stress levels (Table 1). An almost linear decrease in DM content throughout the storage period was observed. However, DM content increased as water deficit increased; this could be attributed to physiological changes induced by water stress during the bulb development stage. DM content increased during the latter phase of the storage (8 months onwards) as a result of the higher rate of moisture loss in onion bulbs produced under no (IW: CPE 1.0–0.85) and low (IW: CPE 0.84–0.70) stress conditions. Medium water stress (IW: CPE 0.69–0.40) should be preferred for bulb storage, as it allows adequate DM content and sustainable yields. Yields reduced drastically under severe water stress (IW: CPE 0.39–0.10). A similar reduction in DM content was earlier reported in onion by Sharma and Lee [6] irrespective of different storage temperatures and by Biswas et al. [16] with increasing levels of irrigations. These results clearly indicate the influence of moisture availability during different growth stages of onion on post-harvest storage. GRs also helped to improve accumulation of DM contents (7.42–11.51%) over control (6.86–10.45%). SB and TU reduced the decrease in DM content with storage period, indicating the role of

GRs in enhancing the storage quality of onions. These results endorse earlier reports on the benefits of GRs and possible mechanisms of translocation of photoassimilates which enhance DM contents under water deficits [17]. Keen optimization of similar key determinants responsible for inducing physicochemical changes in onion during long-term storage can potentially pave the way towards ensuring a sustained supply of high-quality onion bulbs during the lean season.

Table 1. Changes in the dry matter (%) of onion bulbs kept for long-term storage (9 months) under different GRs and water stress levels.

Storage Period (T)	Growth Regulators (GRs)					Water Stress (WS)			
	PN [#]	SB	TU	GA	no GRs	No Stress	Low	Medium	Severe
0	11.04	10.54	10.87	10.67	10.45	10.15	10.47	10.88	11.34
1	11.40	10.73	11.19	10.86	10.40	10.28	10.65	11.17	11.57
2	11.51	10.91	11.28	10.70	10.07	10.47	10.68	11.00	11.39
3	10.9	10.55	10.67	9.88	9.32	9.73	10.07	10.46	10.77
4	9.91	9.85	9.57	9.02	8.45	8.84	9.04	9.57	9.98
5	9.19	9.54	8.98	8.04	7.57	8.29	8.49	8.87	9.00
6	8.19	9.13	8.79	7.15	6.69	7.94	7.99	8.00	8.02
7	7.78	8.75	8.14	7.20	6.84	7.70	7.61	7.79	7.86
8	7.64	8.76	7.79	7.40	6.95	7.97	7.79	7.51	7.55
9	7.67	8.74	8.02	7.42	6.86	8.18	7.84	7.57	7.35
LSD (p = 0.05)	GRs	0.28 *	T		0.37 *	GRs × T	0.83 *	GRs × WS × T	1.66 [ns]
	WS	0.08 *	GRs × WS		0.19 [ns]	WS × T	0.74 [ns]		

[#] PN—potassium nitrate; SB—sodium benzoate; TU—thio-urea; and GA—gibberellic acid. At the p = 0.05 level, * indicate the significant and [ns] non-significant differences in mean values of GRs, WS, T, and their interactions.

Weight loss during storage was significantly affected by GRs as well as by water stress (Figure 3A). In general, weight loss percentage increased as water stress and storage time increased. The most evident effect was observed after nine months of storage in bulbs produced under severe water stress, where weight loss (37.2%) was more than double that of no stressed (17.7%) (Figure 3B). Water loss induces bulb shrinkage, negatively affecting consumer acceptance. Overall, water stress had a detrimental effect on storage duration since it enhanced shrinkage and sprouting initiation, consequently resulting in rapid bulb decline and quality degradation. This result agrees with the finding of Fatideh and Asil [18]. During nine months of storage, SB, TU, PN, and GA were found to effectively reduce bulb weight losses to 17.3, 23.9, 26.4 and 32.2%, respectively, as compared to control (36.2%) (Figure 3B). These results indicate that GRs decreased tissue permeability, thereby reducing the rate of water loss. This led to delayed bulb sprouting and physiological weight loss. These findings are further strengthened by a previous study of Srividya et al. [19], who reported that SB and GA_3 significantly maintain firmness, reduce weight loss, and thereby enhance shelf life of tomatoes.

3.2. Rehydration Quality Characteristics

Rehydration ratio (RR) was significantly affected by GRs, water stress, and storage period (Table 2). The interaction among these factors was also significant ($p \leq 0.05$). During nine-month storage, RR varied continuously in a similar pattern at all water stress levels. In the 1st month, RR increased and then decreased consistently up to 6 months; thereafter, it increased in the subsequent months. Bulbs produced under water stress conditions (IW: CPE 0.84–0.10) gave higher RR (4.27–6.90) as compared to full irrigation bulbs (4.10–5.20). These changes in storage are correlated with DM content, which increased as water deficit increased, and thereby induced positivity in the rehydration quality of onions in a stressed environment. However, visible sprouting was observed after 6 months of storage; this led to a continuous increase in RR at the latter stage of onion storage. The RR of bulbs produced under full irrigation was lower than that in bulbs produced under water deficits

due to higher irreversible cellular rupture and dislocation during drying. Such damaged cells lose their structural integrity during storage due to the moisture stress of the storage environment, and thereby decrease the rehydration capability of the stored material [20]. Further GRs helped to improve the RR (4.42–6.30) over control (4.10–5.50) throughout the storage period. The minimum 9.68% reduction in RR with SB indicates the role of GRs in improving bulb quality during storage. SB inhibits the production of ethylene by interfering with aminocyclopropane-1 carboxylic acid's conversion to ethylene, thereby improving the quality and shelf life of stored fruits [19]. Huanga and Jianga [21] report that GRs, such as chlorfenuron and GA_3, inhibit respiration rate, retard surface colour change, and resist the fungal infection of stored banana fruit and broccoli. GRs also stimulate cell growth and facilitate plant regeneration, increasing yield and fruit quality in stressed environments [22].

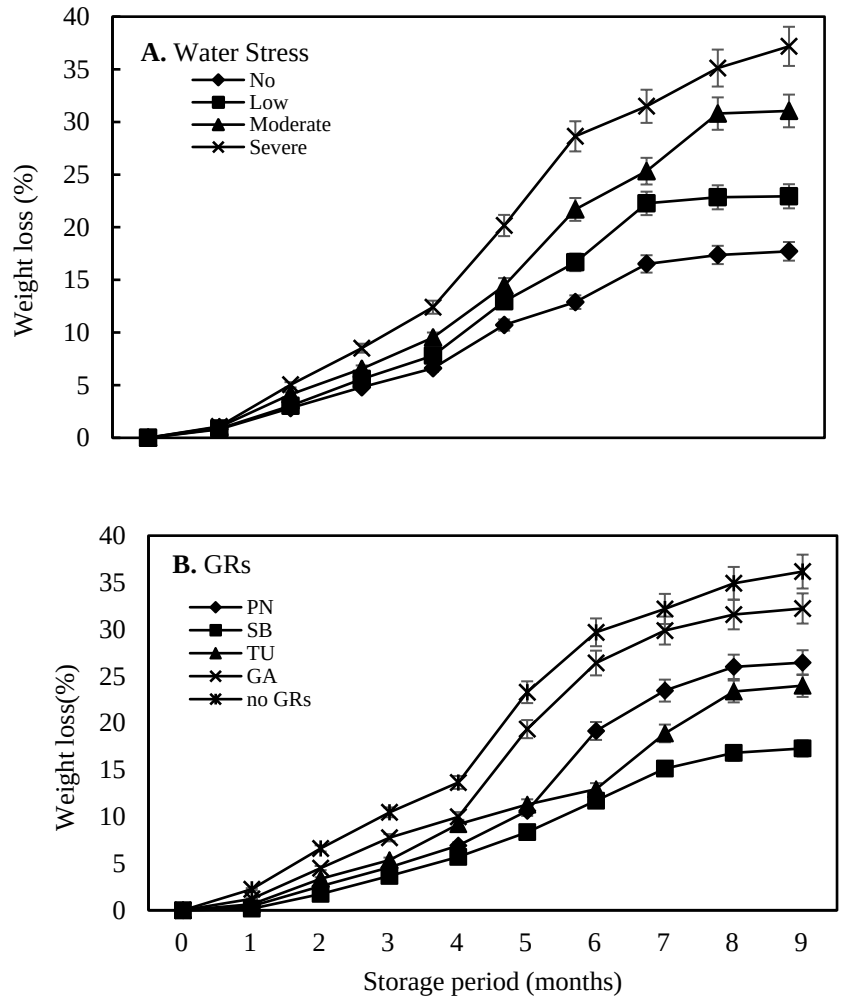

Figure 3. Effect of water stress on the weight loss of onion bulbs (**A**) and growth regulators (GRs) (**B**) across nine months of storage.

Table 2. Influence of different GRs and water stress levels on rehydration ratio (RR) of onion during 9 months of storage period.

Storage Period (T)	0	1	2	3	4	5	6	7	8	9
Growth regulators (GRs)										
PN [#]	6.30 [a]	6.20 [a]	6.08 [a]	5.86 [a]	5.43 [a]	5.12 [b]	4.77 [b]	4.70 [b]	4.87 [bc]	4.89 [b]
SB	5.76 [d]	5.65 [d]	5.55 [d]	5.46 [b]	5.33 [ab]	5.32 [a]	5.12 [a]	5.12 [a]	5.18 [a]	5.20 [a]
TU	6.15 [b]	6.03 [b]	5.90 [b]	5.73 [a]	5.23 [bc]	5.02 [b]	4.62 [c]	4.90 [b]	5.02 [ab]	4.98 [b]
GA	5.95 [c]	5.76 [c]	5.63 [c]	5.54 [b]	5.13 [c]	4.82 [c]	4.42 [d]	4.54 [bc]	4.68 [cd]	4.72 [c]
No GRs	5.50 [e]	5.43 [e]	5.31 [e]	5.06 [c]	4.67 [d]	4.52 [d]	4.10 [e]	4.34 [c]	4.49 [d]	4.51 [d]
Water stress (WS)										
No stress	5.20 [d]	5.06 [d]	4.96 [d]	4.81 [d]	4.54 [c]	4.40 [d]	4.10 [d]	4.29 [c]	4.42 [d]	4.43 [d]
Low	5.50 [c]	5.44 [c]	5.25 [c]	5.04 [c]	4.66 [c]	4.55 [c]	4.27 [c]	4.47 [c]	4.62 [c]	4.62 [c]
Medium	6.10 [b]	5.95 [b]	5.81 [b]	5.67 [b]	5.19 [b]	4.95 [b]	4.61 [b]	4.80 [b]	4.95 [b]	4.96 [b]
Severe	6.90 [a]	6.77 [a]	6.74 [a]	6.59 [a]	6.26 [a]	5.95 [a]	5.44 [a]	5.34 [a]	5.42 [a]	5.42 [a]
LSD (p = 0.05)	GRs	0.19 *		GRs × WS		0.11 *				0.42 [ns]
	WS	0.05 *		GRs × T		0.21 *		GRs × WS × T		
	T	0.09 *		WS × T		0.19 *				

[#] PN—potassium nitrate; SB—sodium benzoate; TU—thio-urea; and GA—gibberellic acid. At the p = 0.05 level, * indicate the significant and [ns] non-significant differences in mean values of GRs, WS, T, and their interactions. DMRT test ($p \leq 0.05$) results indicate that values in a column followed by similar letters are not significantly different.

3.3. Total Sugar Contents (TSS) as Affected by Water Stress and GRs

Total soluble sugar content (TSS) in bulb tissue was significantly influenced by both GRs and water stress levels during storage (Table 3). Under all the water stress levels, TSS increased initially from 31.74–34.80 to 33.91–40.13 mg/g FW for the first three months, remained stable in the fourth month (32.77–39.69 mg/g FW); and then decreased consistently (25.11–33.46 mg/g FW) during the subsequent months. The characteristic initial upward and constant trend of TSS could be related both to the breakup of dormancy, which affects the metabolism of sugars [23], and to the intensification of fructan hydrolysis before the initiation of sprouting [24], respectively. According to Chope et al. [25], onset of inner and visible sprouting after 6 months of storage markedly influences the overall bulb–sugar accumulation since sugars are metabolized to provide energy for the growing sprout, thereafter continuously decreasing the TSS content during the subsequent months. However, these findings contradict those of Rutherford and Whittle [26], who found that total sugar content remained remarkably constant throughout storage. Sharma and Lee [6] looked at the continuous decrease in sugar content of bulbs during long-term storage and how it relates to internal sprouting and deteriorating onion quality. In the present study, TSS of onion bulbs increased as water stress increased throughout the entire storage period. Sugars were found to serve as osmolytes during water stress, therefore, the rise in bulb sugar could be credited to the physiological response of the crop to water stress conditions, which ultimately sunk into the developing bulb. On the contrary, Mohammad and Moazzam [18] reported that TSS in onions increased with the increase in irrigation. The GRs had a beneficial effect in improving TSS content during storage. PN and TU performed better in the initial 5 months while SB maintained higher TSS at a later storage period, suggesting a positive relation of GR application in storage with taste and sweetness, therefore creating better consumer acceptance. In contrast, higher root elongation and sprouting was noticed in the last three storage months in bulbs treated with GA. This reduced TSS (28.14–26.10 mg/g FW) significantly, even at a lower level than control (28.59–27.60 mg/g FW). GA generally induces physiological changes that initiate the production of hydrolytic enzymes, which in turn break down the starch reserve in the bulbs into simple sugars that are utilized for root and shoot growth and development [8]. Thus, these results signify the critical role of the time–treatment relationship, particularly when growth regulators are

applied during storage. The initial increase in TSS in GRs-treated onion bulbs was due to the accumulation of sugar as a consequence of starch hydrolysis. Whereas later on, TSS decreased due to the respiration consumption of sugar during storage [27]. Even though these results permit much insight into the sugar metabolism status of onion bulbs during prolonged storage, current knowledge regarding the effects of GRs and storage period on the TSS of onion bulbs is scarce. Nevertheless, the present study reveals the role of GRs' exogenous application in improving TSS contents throughout the storage period, thereby enhancing the storage quality of onions.

Table 3. Sugar content (mg/g FW) of onions as influenced under different GRs and water stress levels during 9 months of storage period.

Storage Period (T)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Growth regulators (GRs)										
PN [#]	33.90 ^{a}	35.31 ^{a}	37.22 ^{a}	38.42 ^{a}	37.76 ^{a}	36.82 ^{a}	33.27 ^{ab}	31.65 ^{ab}	30.63 ^{ab}	30.20 ^{b}
SB	32.64 ^{cd}	33.38 ^{cd}	34.88 ^{bc}	35.83 ^{d}	35.95 ^{c}	35.41 ^{b}	34.11 ^{a}	32.74 ^{a}	31.55 ^{a}	31.34 ^{a}
TU	33.60 ^{ab}	34.80 ^{ab}	36.67 ^{a}	37.52 ^{b}	36.79 ^{b}	34.68 ^{b}	32.08 ^{b}	30.21 ^{b}	29.68 ^{b}	29.21 ^{c}
GA	33.16 ^{bc}	34.12 ^{bc}	35.49 ^{b}	36.59 ^{c}	34.83 ^{d}	33.56 ^{c}	30.44 ^{c}	28.14 ^{c}	26.80 ^{d}	26.10 ^{e}
No GRs	32.39 ^{d}	32.87 ^{d}	34.17 ^{c}	35.36 ^{d}	34.26 ^{d}	32.66 ^{d}	29.60 ^{d}	28.59 ^{c}	28.20 ^{c}	27.60 ^{d}
Water stress (WS)										
No stress	31.74 ^{d}	32.32 ^{d}	33.55 ^{d}	33.91 ^{d}	32.77 ^{d}	32.16 ^{d}	28.95 ^{d}	27.29 ^{d}	25.60 ^{d}	25.11 ^{d}
Low	32.38 ^{c}	33.19 ^{c}	34.59 ^{c}	35.10 ^{c}	34.12 ^{c}	33.38 ^{c}	29.89 ^{c}	28.61 ^{c}	27.50 ^{c}	26.79 ^{c}
Medium	33.65 ^{b}	34.69 ^{b}	36.41 ^{b}	37.83 ^{b}	37.11 ^{b}	35.54 ^{b}	32.93 ^{b}	30.98 ^{b}	30.48 ^{b}	30.19 ^{b}
Severe	34.80 ^{a}	36.21 ^{a}	38.22 ^{a}	40.13 ^{a}	39.69 ^{a}	37.43 ^{a}	35.81 ^{a}	34.21 ^{a}	33.89 ^{a}	33.46 ^{a}
LSD (p = 0.05)	GRs	0.46 *		GRs × WS		0.52 *				3.36 ^{ns}
	WS	0.23 *		GRs × T		1.68 *		GRs × WS × T		
	T	0.75 *		WS × T		1.50 *				

[#] PN—potassium nitrate; SB—sodium benzoate; TU—thio-urea; and GA—gibberellic acid. At the p = 0.05 level, * indicate the significant and ^{ns} non-significant differences in mean values of GRs, WS, T, and their interactions. DMRT test ($p \leq 0.05$) results indicate that values in a column followed by similar letters are not significantly different.

3.4. Periodic Changes in Protein Contents during Storage

During the initial stages of storage, the protein content in bulbs produced under varied water deficits increased slightly from 9.21–12.24 to 9.76–13.05 mg/g FW, then declined gradually (6.71–11.13 mg/g FW) for up to 6 months due to dormancy and initiation of internal sprouts (Table 4). Interestingly, after 7 months, the protein content increased again (7.66–12.02 mg/g FW). Overall protein content varied in a similar, but quite irregular pattern throughout the storage, with reduced amplitude and a period of fluctuations in water deficits. The reason could be the decrease in carbohydrate content during storage, mostly due to increased respiration, which consequently resulted in higher nitrogen and protein content in the dry matter [27].

Table 4. Periodic changes in protein (mg/g FW) of onions under different GRs and water stress levels during 9 months of storage period.

Storage Period (T)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Growth regulators (GRs)										
PN [#]	11.01 ^{a}	11.53 ^{a}	11.68 ^{a}	11.16 ^{a}	10.45 ^{a}	9.64 ^{a}	9.20 ^{b}	9.12 ^{a}	9.02 ^{b}	9.17 ^{c}
SB	10.10 ^{d}	10.55 ^{b}	10.75 ^{cd}	10.25 ^{b}	9.92 ^{b}	9.80 ^{a}	9.60 ^{a}	9.42 ^{a}	9.58 ^{a}	9.79 ^{b}

Table 4. *Cont.*

Storage Period (T)	0	1	2	3	4	5	6	7	8	9
TU	10.75 ^{b}	11.28 ^{a}	11.42 ^{ab}	10.79 ^{ab}	10.30 ^{a}	9.25 ^{b}	8.84 ^{c}	8.22 ^{b}	8.56 ^{c}	8.56 ^{d}
GA	10.42 ^{c}	10.71 ^{b}	11.01 ^{bc}	10.41 ^{b}	9.21 ^{c}	8.51 ^{c}	8.10 ^{d}	8.35 ^{b}	9.72 ^{a}	10.36 ^{a}
No GRs	10.08 ^{d}	10.35 ^{b}	10.32 ^{d}	9.72 ^{c}	8.67 ^{d}	8.21 ^{c}	7.37 ^{e}	7.72 ^{c}	8.35 ^{c}	9.03 ^{c}
Water stress (WS)										
No stress	9.21 ^{d}	9.67 ^{a}	9.76 ^{c}	9.16 ^{c}	8.25 ^{d}	7.51 ^{d}	6.71 ^{d}	6.62 ^{d}	7.31 ^{d}	7.66 ^{d}
Low	9.72 ^{c}	10.13 ^{c}	10.12 ^{c}	9.55 ^{c}	8.87 ^{c}	8.02 ^{c}	7.57 ^{c}	7.21 ^{c}	7.89 ^{c}	7.99 ^{c}
Medium	10.70 ^{b}	11.05 ^{b}	11.22 ^{b}	10.71 ^{b}	9.93 ^{b}	9.29 ^{b}	9.06 ^{b}	9.09 ^{b}	9.28 ^{b}	9.85 ^{b}
Severe	12.24 ^{a}	12.67 ^{a}	13.05 ^{a}	12.46 ^{a}	11.78 ^{a}	11.49 ^{a}	11.13 ^{a}	11.37 ^{a}	11.71 ^{a}	12.02 ^{a}
LSD (p = 0.05)	GRs	0.18 *		GRs × WS		0.24 *				1.27 ^{ns}
	WS	0.11 *		GRs × T		0.63 *		GRs × WS × T		
	T	0.28 *		WS × T		0.57 *				

$^{\#}$ PN—potassium nitrate; SB—sodium benzoate; TU—thio-urea; and GA—gibberellic acid. At the p = 0.05 level, * indicate the significant and ^{ns} non-significant differences in mean values of GRs, WS, T, and their interactions. DMRT test ($p \leq 0.05$) results indicate that values in a column followed by similar letters are not significantly different.

The protein content increased in GR-treated bulbs throughout the storage period. Statistical analysis showed that GRs, water stress, storage time, and their interactions significantly affected protein content ($p \leq 0.05$). These might be due to the inhibition of the proteolytic activity during storage. SB showed a minimum (0.97%) decreased amplitude of protein content during the whole storage, followed by 2.66%, 2.87%, 2.91% and 2.98% in PN, TU, GA, and control, respectively. In previous studies, the protective mechanism of SB in stabilising salt, soluble nitrogen, and protein denaturation during storage [28] and the role of K in protein synthesis under water deficits [29] were well demonstrated. Further, at the physiological level, TU coordinates regulation of the plant source-to-sink relationship, thereby enhancing translocation of metabolites into proteins [17]. Thus, our findings strongly suggest that GRs may play a role in the overall maintenance of high protein content and better physiological status of onion bulbs in storage. Increased proteins and amino acid content were reported in many crop plants as a response to water deficit; moreover, the influence of GRs on protein content during the vegetative stage is also reported [30]. Therefore, selection of the appropriate GR according to the water availability during the bulb production can be crucial to maintain nutritional quality of onions in terms of proteins during long-term storage.

3.5. Influence of GRs and Water Stress on Total Phenolics (TP) during Storage

Onion contains high levels of phenolic compounds having antioxidant properties besides beneficial effects against different degenerative pathologies. The effect of temperature and water stress on the total phenolics contents (TP) of bulb tissue were studied previously [8]. However, to our knowledge, no reports are available on changes in TP during storage of onions produced at different water stress levels and GR practices. In our study, GRs, water deficits, and storage time had a significant effect ($p \leq 0.05$) on TP. TP increased initially during the first month; thereafter, a decreasing trend was recorded up to the fifth month, followed by a slight increase to almost stable trend at different water stress levels in the following months (Figure 4A). This might be due to coinciding enzymatic activities as a result of physiological alterations. Similar increases and decreases in TP during storage under controlled conditions were reported by Benkeblia and Shiomi [31]. These findings indicate that physiological factors, such as dormancy break and sprouting could be the controlling factors of TP; the role of TP in cellular homeostasis, particularly in the management of oxidative stress, signalling, and dormancy, is well known [31]. At no stress (IW: CPE 1.00) TP was 2.01–3.24 mg GAE/g FW, while as the levels of water stress throughout the storage increased, TP ranged from 2.39 to 6.02 mg GAE/g FW (Figure 4B). This could be attributed to higher levels of water deficit-imposed oxidative stress, where

the phenolics fraction could contribute to the non-enzymatic management of superoxide radicals. Figure 1b depicts a significant rise in TP under the influence of GR treatments. The TP in onion bulbs decreased significantly (15.23–34.33%) for up to 5 months, but in an irregular pattern for all GRs, and remained stable for SB, PN, and TU between the 6th and 9th months of storage. Further, TU and PN enhanced TP up to 6 months, while SB outperformed to maintain TP throughout storage. Thus, higher concentration of TP indicated that GRs are responsible for extending the dormant state of the onion, and thereby the storage quality [31]. Additionally, the highest TP concentration was observed in GA, followed by the control (no GR) at the later phase (8th and 9th months) of storage, owing to higher sprouting and complete bulb decay. This study thus signifies the probable contribution of GRs in reducing storage losses due to water deficit governed by oxidative stress in onions. Additionally, greater bulb size produced under no stress conditions imposes limits on TP. We identified a strategy of integrative use of GRs, along with deficit irrigation, where TP concentrations can be improved without much post-harvest storage losses in onions.

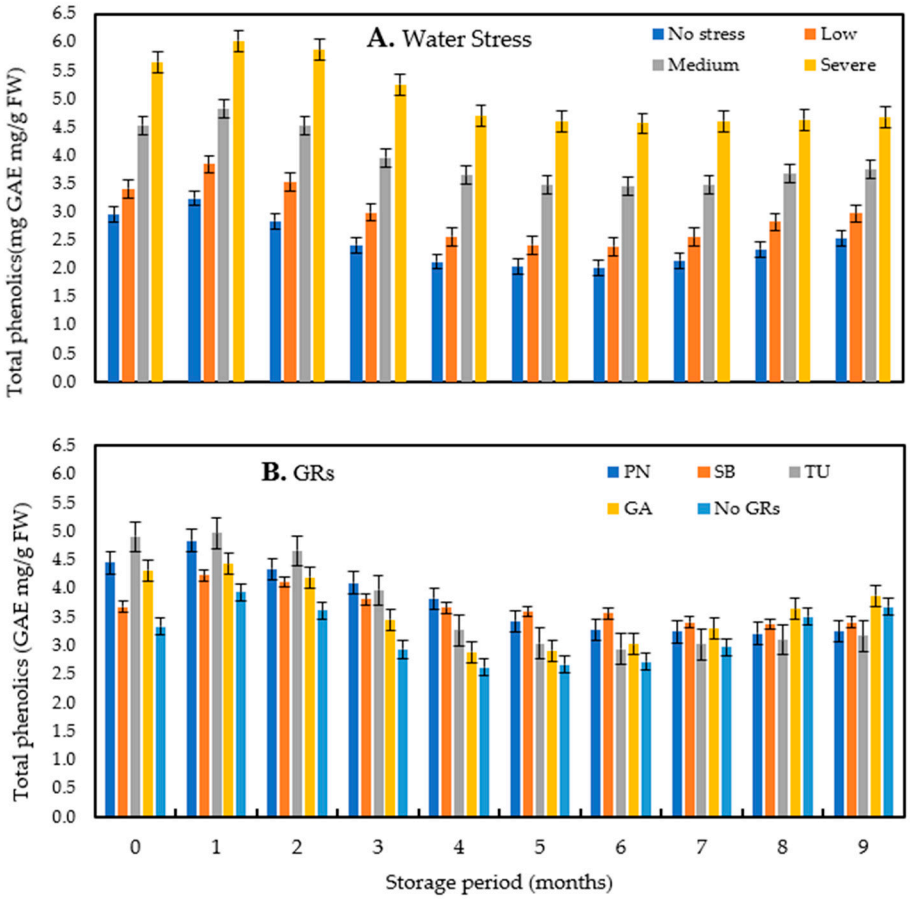

Figure 4. Effect of water stress on total phenolics (**A**) and growth regulators (GRs) (**B**) across nine months of storage.

3.6. Antioxidant Enzymes Activity (AOA)

The activities of antioxidant enzymes viz. SOD and POD from stored onion bulbs were analyzed, and the results are illustrated in Tables 5 and 6. SOD and POD activities were significantly higher in water deficit treatments during the entire storage period. In a real sense, water deficit imposes oxidative stress at the cellular level under which SOD scavenges superoxide radicals enzymatically into O_2 and H_2O_2. Further hydrogen peroxide is detrimental, but at a lower level, because it is removed by POD, catalase (CAT), and other enzymes. Thus, it is possible to hypothesise that a lack of water increases H_2O_2 concentrations in stored bulbs, and thus total phenolics and overall enzymatic defence

systems. Furthermore, moderate and severe stress increases the synthesis of secondary metabolites, which activates AOA activity. Despite cultivars being tolerant, the literature reveals increased levels of AOA, such as SOD and APX in plants under stress conditions due to limited water uptake [2]. Under all the water stress treatments, the SOD and POD activity followed a similar pattern with temporal differences. SOD activity increased from 139.8–167.6 to 179.2–251.5 U/mg protein during the initial 4 months, then decreased continuously (172.3–221.7 U/mg protein) up to 7 months and later at the end of storage it maintained 177.1–226.9 U/mg protein (Table 5). Similarly, POD activity improved significantly from 69.6–80.9 to 91.8–111.8 U/100 g FW in the first 3 months and later decreased to 70.6–89.7 U/100 g FW in the next 4 months, and remained stable or increased slightly in some cases during the last 2 months of storage (Table 6). Thus, a first increase in AOA might be an indicator of dormancy break, which leads to initiation of internal sprouting; while a further decrease in AOA coincides with the onset of sprout from the bulbs. Previously, Sharma et al. [10] reported a similar trend in AOA, where POD activity was monitored in onion bulbs stored at 25 °C for 9 months. Total concentrations of the SOD and POD were higher as compared to the fresh samples during the storage at all water stress and GR treatments. These results corroborate those of Kevers et al. [32], indicating that the antioxidant activity of onions increased 10 times after 23 days of storage subsequent to purchase from the market. Further, foliar application of GRs also successfully induced the up-regulation of the AOA activity throughout the storage, indicating a clear influence of the growth regulators applied before the harvesting stage on the produce. Overall GRs, water deficits, storage time, and their interactions had a significant effect on AOA activities ($p \leq 0.05$). SOD and POD activities from the bulb tissue ranged between 148.62–232.81 U/mg protein and 74.62–111.03 U/100 g FW under the influence of GRs, whereas their values declined (144.26–209.56 U/mg protein and 71.25–93.53 U/100 g FW) in absence of GRs during the entire storage period. The maximum increase in SOD and POD was observed with PN, followed by TU, irrespective of water deficits. As similar to TP, AOA activity in GA and no GR treatments increased at a later phase of storage, indicating that the TP can be correlated with AOA activities. Similar correlation was reported by Santas et al. [33] between the TP and AOA of stored onions. The findings indicate that exogenous application of GRs during storage stimulated and regulated antioxidant enzymes, allowing for better management of water deficit-mediated cellular oxidative stress and thus protecting the cells' vital compartments from the deleterious effects of reactive oxygen species (ROS). The mechanism of GR-mediated up-regulation of antioxidant enzyme activity was reported in other crops as well [34]. Thus, our results have implications for water budgeting, enhancement of yield, and post-harvest storage quality of onions cultivated under water deficit environmental conditions.

Table 5. Variation in SOD (unit/mg protein) of onions under different GRs and water stress levels during 9 months of storage.

Storage Period (T)	0	1	2	3	4	5	6	7	8	9
					Growth regulators (GRs)					
PN #	158.20 ^{a}	180.65 ^{a}	198.11 ^{a}	231.25 ^{a}	232.81 ^{a}	217.53 ^{a}	203.75 ^{a}	190.12 ^{bc}	195.35 ^{b}	199.09 ^{bc}
SB	148.62 ^{bc}	161.54 ^{cd}	183.90 ^{b}	206.63 ^{c}	213.50 ^{cd}	207.12 ^{b}	199.31 ^{a}	197.80 ^{a}	195.48 ^{b}	197.23 ^{bc}
TU	155.42 ^{a}	173.45 ^{b}	192.61 ^{a}	219.75 ^{b}	226.55 ^{b}	213.23 ^{ab}	196.30 ^{ab}	191.12 ^{abc}	190.36 ^{b}	195.48 ^{c}
GA	152.74 ^{ab}	165.53 ^{c}	185.24 ^{b}	211.16 ^{c}	218.27 ^{c}	198.30 ^{c}	189.25 ^{bc}	194.51 ^{ab}	204.83 ^{a}	206.70 ^{a}
No GRs	144.26 ^{c}	157.23 ^{d}	177.71 ^{c}	200.25 ^{d}	209.56 ^{d}	191.45 ^{d}	185.38 ^{c}	185.47 ^{c}	202.30 ^{a}	203.64 ^{ab}

Table 5. *Cont.*

Storage Period (T)	0	1	2	3	4	5	6	7	8	9
Water stress (WS)										
No stress	139.83 ^{d}	152.52 ^{d}	160.32 ^{d}	179.24 ^{d}	190.85 ^{d}	171.20 ^{d}	172.53 ^{d}	170.30 ^{d}	179.74 ^{d}	177.13 ^{d}
Low	147.41 ^{c}	159.56 ^{c}	174.81 ^{c}	197.70 ^{c}	203.56 ^{b}	190.27 ^{c}	182.20 ^{c}	181.56 ^{c}	187.58 ^{c}	191.27 ^{c}
Medium	152.52 ^{b}	171.40 ^{b}	196.45 ^{b}	226.80 ^{b}	231.45 ^{b}	219.12 ^{b}	202.70 ^{b}	198.20 ^{b}	202.12 ^{b}	206.41 ^{b}
Severe	167.63 ^{a}	187.25 ^{a}	218.47 ^{a}	251.50 ^{a}	254.70 ^{a}	241.50 ^{a}	221.75 ^{a}	217.12 ^{a}	221.20 ^{a}	226.90 ^{a}
LSD (p = 0.05)	GRs	3.16 *		GRs × WS		2.92 *				16.24 ^{ns}
	WS	1.30 *		GRs × T		8.12 *		GRs × WS × T		
	T	3.63 *		WS × T		7.26 *				

$^{\#}$ PN—potassium nitrate; SB—sodium benzoate; TU—thio-urea; and GA—gibberellic acid. At the p = 0.05 level, * indicate the significant and ^{ns} non-significant differences in mean values of GRs, WS, T, and their interactions. DMRT test ($p \leq 0.05$) results indicate that values in a column followed by similar letters are not significantly different.

Table 6. Changes in POD (unit/100 g FW) of onions under different GRs and water stress levels during 9 months of storage.

Storage Period (T)	0	1	2	3	4	5	6	7	8	9
Growth regulators (GRs)										
PN $^{\#}$	79.38 ^{a}	97.45 ^{a}	111.03 ^{a}	104.41 ^{a}	94.04 ^{a}	88.44 ^{a}	84.72 ^{a}	82.57 ^{a}	84.18 ^{b}	85.07 ^{bc}
SB	73.25 ^{cd}	87.61 ^{cd}	95.15 ^{d}	93.11 ^{c}	85.32 ^{c}	83.79 ^{ab}	80.68 ^{b}	81.28 ^{a}	82.42 ^{bc}	83.24 ^{bc}
TU	76.56 ^{b}	93.53 ^{b}	106.14 ^{b}	100.37 ^{b}	90.24 ^{b}	86.69 ^{a}	79.49 ^{b}	80.14 ^{ab}	80.41 ^{c}	82.74 ^{c}
GA	74.62 ^{bc}	89.54 ^{c}	99.48 ^{c}	95.54 ^{c}	86.23 ^{c}	82.43 ^{b}	76.59 ^{c}	81.43 ^{a}	86.81 ^{a}	89.58 ^{a}
No GRs	71.25 ^{d}	84.96 ^{d}	93.53 ^{d}	91.22 ^{d}	81.56 ^{d}	77.12 ^{c}	74.87 ^{c}	77.57 ^{b}	84.54 ^{ab}	87.12 ^{ab}
Water stress (WS)										
No stress	69.63 ^{d}	82.92 ^{d}	91.84 ^{d}	86.45 ^{d}	78.50 ^{d}	75.48 ^{d}	70.56 ^{d}	71.15 ^{d}	73.12 ^{d}	72.54 ^{c}
Low	72.89 ^{c}	87.40 ^{c}	96.16 ^{c}	92.42 ^{c}	83.39 ^{c}	80.12 ^{c}	74.63 ^{c}	76.53 ^{c}	78.54 ^{c}	81.33 ^{b}
Medium	76.63 ^{b}	93.64 ^{b}	104.47 ^{b}	101.36 ^{b}	91.16 ^{b}	86.45 ^{b}	82.12 ^{b}	83.48 ^{b}	89.39 ^{b}	92.83 ^{a}
Severe	80.90 ^{a}	98.51 ^{a}	111.80 ^{a}	107.49 ^{a}	96.88 ^{a}	92.71 ^{a}	89.75 ^{a}	91.23 ^{a}	93.61 ^{a}	95.48 ^{a}
LSD (p = 0.05)	GRs	1.63 *		GRs × WS		2.21 ^{ns}		GRs × WS × T		10.20 ^{ns}
	WS	0.99 *		GRs × T		5.10 *				
	T	2.28 *		WS × T		4.56 ^{ns}				

$^{\#}$ PN—potassium nitrate; SB—sodium benzoate; TU—thio-urea; and GA—gibberellic acid. At the p = 0.05 level, * indicate the significant and ^{ns} non-significant differences in mean values of GRs, WS, T, and their interactions. DMRT test ($p \leq 0.05$) results indicate that values in a column followed by similar letters are not significantly different.

3.7. Changes in Pungency (Pyruvic Acid Content) of Stored Onion

The consumer perception of onion pungency depends on an array of organo-sulphur containing volatile compounds (VOCs), such as 1−propenyl cysteine sulphoxide, methyl cysteine sulphoxide, and propyl cysteine sulphoxide generated upon mechanical injury when the bulb cells are disrupted [35]. These compounds then come into contact with the alliinase enzyme that produces pyruvic acid in proportion to the VOCs. Pyruvic acid content can thus be used to predict onion pungency. Onion pungency is affected by many factors, including sulphur-based fertilization, cultivar genotypes, dry matter, abiotic stress environment, and storage [23]. We found a significant rise in the pyruvic acid content of bulb tissue throughout storage for all water stress and GR treatments in this study. The change in pyruvic acid content showed a similar pattern at all water stress levels and was almost stable (23.11–33.77 µmol/g FW) for the initial 5 months, followed by an increase of 29.12–42.15 µmol/g FW in subsequent months, respectively (Figure 5A). This variation is

linked with dormancy and sprouting since the first sprouting was observed at 5 months of storage, which was in agreement with the previous findings of Sharma et al. [10]. The pyruvic acid concentration in GRs-treated onions fluctuated between 25.85 and 32.65 mol/g FW with increase and decrease until 5 months, then increased to 29.97–39.23 mol/g FW after 9 months of storage (Figure 5B). The pyruvic acid content varied with changes in dry matter due to weight loss and tissue dehydration during storage [10]; this could be another possible reason for the increase in pyruvic acid detected under both water deficits and GR treatments after long-term storage. At the initial storage period of 6 months, PN and TU enhanced and maintained the pungency level, while SB performed best at the later phase of storage. This might be due to the role of KNO_3 (PN) as a nitrogen source, and TU as a sulfhydryl group, and their attribution to biosynthesis of amino acids such as cysteine, as well protect metabolites from oxidation [22]. Thus, GRs helped to maintain the pungency level of stored onions almost similar to fresh onions as preferred by the consumer. During the last three months of storage, maximum pyruvic acid content (33.16–40.58 µmol/g FW) was recorded in GA and no GR treatment owing to higher occurrence of sprouting. Overall results show that GRs, water deficits, storage time, and their interactions had a significant effect on pyruvic acid content ($p \leq 0.05$). Pungency being one of the major economic and quality determinants in onions, our results highlight the implication of GR applications in the overall improvement of bulb yields and ensure the post-harvest storage quality of onions for both growers and consumers under water-scarce environments.

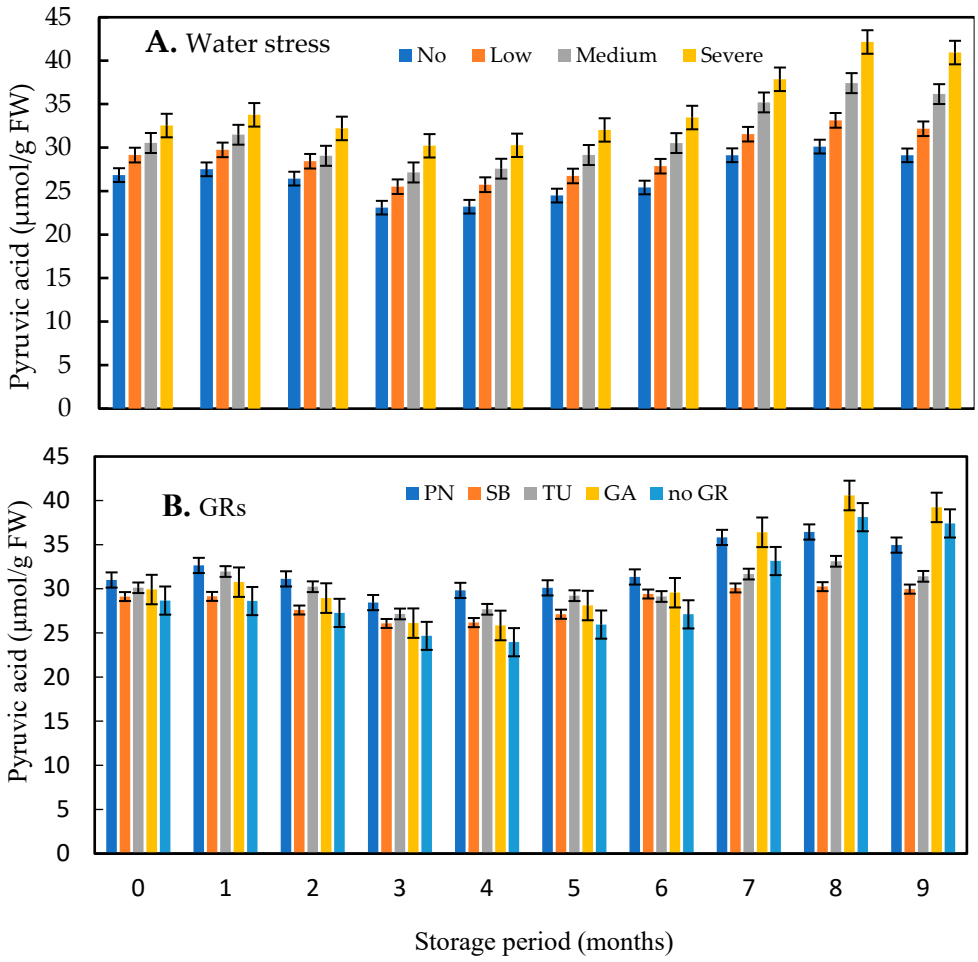

Figure 5. Effect of water stress on pyruvic acid (**A**) and plant bioregulators (**B**) across nine months of storage.

4. Conclusions

Water deficit in onions is a major concern to sustain the bulb yield while maintaining the keeping quality during long-term storage. Water stress and GRs mediated the increase in dry matter, rehydration ratio, TSS, protein content, total phenolics, pyruvic acid, and antioxidant enzymes activities, though in an irregular pattern throughout the storage of 9 months. GRs such as SB, PN, and TU were identified to play a significant role in improving bulb yield, and keeping quality during long-term storage by regulating physiological and metabolic activities of onions grown under water-scarce conditions. Exogenous application of GRs with moderate water stress (IW: CPE 0.69–0.40) is, therefore, suggested as a key strategy to maintain the quality of onion bulbs during long-term storage, while rationalising exogenous GRs for onions grown under severe water stress was considered less advisable for long-term storage owing to higher weight loss (37.2%), sprouting incidences, and reduced bulb size with poor-grade bulbs, irrespective of high physiochemical and functional characteristics. Our results, thus, have a crucial practical application, as storage in cold rooms implies a high cost in the food industry market, especially for bulk products such as onions.

Author Contributions: All the authors made significant contributions to the manuscript. Conceptualization, G.C.W.; methodology, G.C.W. and P.S.K. (Pratapsingh S. Khapte); formal analysis, G.C.W.; writing—original draft preparation, G.C.W.; writing—review and editing, P.S.K. (Pratapsingh S. Khapte), S.K., P.S.K. (Paramsivam Suresh Kumar), P.K. and L.S. Validation— G.C.W., P.S.K. (Pratapsingh S. Khapte), S.K., P.S.K. (Paramsivam Suresh Kumar), P.K. and L.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All relevant data supporting the findings of this study are included in this article. Correspondence and requests for materials should be addressed to G.C.W.

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References

1. Wakchaure, G.C.; Minhas, P.S.; Meena, K.K.; Singh, N.P.; Hegade, P.M.; Sorty, A.M. Growth, bulb yield, water productivity and quality of onion (*Allium cepa* L.) as affected by deficit irrigation regimes and exogenous application of plant bio–regulators. *Agric. Water Manag.* **2018**, *199*, 1–10. [CrossRef]
2. Csiszár, J.; Lantos, E.; Tari, I.; Madosa, E.; Wodala, B.; Vashegyi, Á.; Horváth, F.; Pécsváradi, A.; Szabó, M.; Bartha, B.; et al. Antioxidant enzyme activities in *Allium* species and their cultivars under water stress. *Plant Soil Environ.* **2007**, *53*, 517–523. [CrossRef]
3. Pelter, G.Q.; Mittelstadt, R.; Leib, B.G.; Redulla, C.A. Effects of water stress at specific growth stages on onion bulb yield and quality. *Agric. Water Manag.* **2004**, *68*, 107–115. [CrossRef]
4. Banuu Priya, E.P.; Sinja, V.R.; Alice, R.P.J.S.; Shanmugasundaram, S.; Alagusundaram, K. Storage of onions—A review. *Agric. Rev.* **2014**, *35*, 239–249. [CrossRef]
5. Petropoulos, S.A.; Ntatsi, G.; Fernandes, Â.; Barros, L.; Barreira, J.C.; Ferreira, I.C.; Antoniadis, V. Long-term storage effect on chemical composition, nutritional value and quality of Greek onion landrace 'Vatikiotiko'. *Food Chem.* **2016**, *201*, 168–176. [CrossRef] [PubMed]
6. Sharma, K.; Lee, Y.R. Effect of different storage temperature on chemical composition of onion (*Allium cepa* L.) and its enzymes. *J. Food Sci. Technol.* **2016**, *53*, 1620–1632. [CrossRef]
7. Majid, I.; Dhatt, A.S.; Sharma, S.; Nayik, G.A.; Nanda, V. Effect of sprouting on physicochemical, antioxidant and flavonoid profile of onion varieties. *Int. J. Food Sci. Technol.* **2016**, *51*, 317–324. [CrossRef]
8. Sharma, K.; Ko, E.Y.; Assefa, A.D.; Ha, S.; Nile, S.H.; Lee, E.T.; Park, S.W. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *J. Food Drug Anal.* **2015**, *23*, 243–252. [CrossRef]
9. Yoo, K.S.; Lee, E.J.; Patil, B.S. Changes in flavor precursors, pungency, and sugar content in short-day onion bulbs during 5-month storage at various temperatures or in controlled atmosphere. *J. Food Sci.* **2012**, *77*, 216–221. [CrossRef]

10. Sharma, K.; Lee, Y.R.; Park, S.W.; Nile, S.H. Importance of growth hormones and temperature for physiological regulation of dormancy and sprouting in onions. *Food Rev. Int.* **2016**, *32*, 233–255. [CrossRef]
11. Ilic, Z.; Trajkovic, R.F.; Lazic, S.; Bursic, V.; Sunjka, D. Maleic hydrazide residues in the onion bulbs induce dormancy and hamper sprouting for long periods. *J. Food Agric. Environ.* **2011**, *9*, 113–118.
12. Ranganna, S. *Hand Book of Analysis of Fruit and Vegetable Products*; Tata Mc Graw Hill Co., Ltd.: New Delhi, India, 1992.
13. Yemm, E.W.; Willis, A.J. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **1954**, *57*, 508–514. [CrossRef]
14. Castillo, F.J.; Penel, C.; Greppin, H. Peroxidase release induced by ozone in *Sedum album* leaves. *Plant Physiol.* **1984**, *74*, 846–851. [CrossRef]
15. Teare Ketter, C.A.; Randle, W.M. Pungency assessment in onions. In *Tested Studies for Laboratory Teaching. As-Sociation for Biology Laboratory Education*; Karcher, S.J., Ed.; Association for Biology Laboratory Education: Williamsport, PA, USA, 1998; pp. 177–196.
16. Biswas, S.; Khair, A.; Sarker, P.; Alom, M. Yield and storability of onion (*Allium cepa* L.) as affected by varying levels of irrigation. *Bangladesh J. Agric. Res.* **2010**, *35*, 247–255. [CrossRef]
17. Srivastava, A.K.; Pasala, R.; Minhas, P.S.; Suprasanna, P. Plant bioregulators for sustainable agriculture: Integrating redox signalling as a possible unifying mechanism. *Adv. Agron.* **2016**, *137*, 237–278.
18. Fatideh, M.M.; Asil, M.H. Onion yield, quality and storability as affected with different soil moisture and nitrogen regimes. *South West. J. Hortic. Biol. Environ.* **2012**, *3*, 145–165.
19. Srividya, S.; Reddy, S.S.P.; Sudhavani, V.; Reddy, R. Effect of post-harvest chemicals on fruit physiology and shelf life of tomato under ambient conditions. *Int. J. Agric. Food Sci. Technol.* **2014**, *5*, 99–104.
20. Gupta, M.K.; Sehgal, V.K.; Singh, A.K.; Singh, R.K. Effect of process parameters and storage length on quality of dried cauliflower during storage. *Indian J. Tradit. Know.* **2012**, *11*, 177–184.
21. Huang, H.; Jiang, Y. Effect of plant growth regulators on banana fruit and broccoli during storage. *Sci. Hortic.* **2012**, *145*, 62–67. [CrossRef]
22. Mohamed, E.; Awadi, E.; Mohamed, S.A.; Wahed, A.E. Improvement the growth and quality of green onion (*Allium cepa* L.) plants by some bioregulators in the new reclaimed area at Nobaria Region, Egypt. *New York Sci. J.* **2012**, *5*, 114–120.
23. Chope, G.A.; Terry, L.A.; White, P.J. The effect of 1-methylcyclopropene (1-MCP) on the physical and biochemical characteristics of onion cv. SS1 bulbs during storage. *Postharvest Biol. Technol.* **2007**, *44*, 131–140. [CrossRef]
24. Benkeblia, N.; Varoquaux, P.; Shiomi, N.; Sakai, H. Storage technology of onion bulbs c.v. Rouge Amposta: Effects of irradiation, maleic hydrazide and carbamate isopropyl, N-phenyl (CIP) on respiration rate and carbohydrates. *Int. J. Food Sci. Technol.* **2002**, *37*, 169–175. [CrossRef]
25. Chope, G.A.; Terry, L.A.; White, P.J. The effect of the transition between controlled atmosphere and regular atmosphere storage on bulbs of onion cultivars SS1, Carlos and Renate. *Postharvest Biol. Technol.* **2007**, *44*, 228–239. [CrossRef]
26. Rutherford, P.P.; Whittle, R. The carbohydrate composition of onions during long term cold storage. *J. Hortic. Sci.* **1982**, *57*, 349–356. [CrossRef]
27. Patil, S.; Chillawar, R.G.; Viradia, R.R. Effect of post-harvest treatments on biochemical changes of Mango cv. Kesar fruit during storage. *Biolife* **2014**, *2*, 1262–1267.
28. Kedar, J.G.; Pagarkar, A.U.; Shingare, P.E.; Bhosale, B.P.; Shinde, K.M.; Kulkarni, G.N. Effect of sodium benzoate on the shelf life of vacuum packed Catla fish steaks stored at chilled temperature. *Biosci. Biotechnol. Res. Commun.* **2016**, *9*, 421–427.
29. Marschner, P. *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Elsevier: Amsterdam, The Netherlands, 2012.
30. Mani, F.; Bettaieb, T.; Zheni, K.; Doudech, N.; Hannachi, C. Effect of thiourea on yield and quality of potato (*Solanum tuberosum* L.). *J. Stress Physiol. Biochem.* **2013**, *9*, 87–95.
31. Benkeblia, N.; Shiomi, N. Chilling effect on soluble sugars, respiration rate, total phenolics, peroxidase activity and dormancy of onion bulbs. *Sci. Agric.* **2004**, *61*, 281–285. [CrossRef]
32. Kevers, C.; Falkowski, M.; Tabart, J.; Defraigne, J.-O.; Dommes, J.; Pincemail, J. Evolution of antioxidant capacity during storage of selected fruits and vegetables. *J. Agric. Food Chem.* **2007**, *55*, 8596–8603. [CrossRef]
33. Santas, J.; Carbo, R.; Gordon, M.; Almajano, M. Comparison of the antioxidant activity of two Spanish onion varieties. *Food Chem.* **2008**, *107*, 1210–1216. [CrossRef]
34. Hassanein, R.A.; Amin, A.A.E.; Rashad, E.M.; Ali, A. Effect of thiourea and salicylic acid on antioxidant defense of wheat plants under drought stress. *Int. J. Chem. Techol. Res.* **2015**, *7*, 346–354.
35. Gallina, P.M.; Cabassi, G.; Maggioni, A.; Natalini, A.; Ferrante, A. Changes in the pyruvic acid content correlates with phenotype traits in onion clones. *Aust. J. Crop Sci.* **2012**, *6*, 36–40.