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MITIGATION OF DROUGHT STRESS IN SPINACH USING INDIVIDUAL AND COMBINED APPLICATIONS OF SALICYLIC ACID AND POTASSIUM

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Abstract

Drought stress is a major constraint impairing crops growth around the globe. It not only decreases the growth of crops but also converted cultivatable land into barren non-cultivatable area. The salicylic acid (SA) and recommended potassium (K) application have the potential to alleviate the adverse effects of drought stress on crops. Current greenhouse pot experiment was conducted to investigate the influence of various levels of SA and K on spinach growth under drought stress. There were three levels of SA i.e., 0 micro Molar (µM), 300 µM and 600 µM and K i.e., control (0), half recommended dose (34.5 mg / 5 kg soil) and full recommended dose (69.0 mg / 5kg). Results showed that drought stress significantly decreased shoot and root, lengths and dry weights, chlorophyll a and b, total chlorophyll and nutrients uptake. It also significantly increased electrolyte leakage in spinach leaves. However, application of SA600 + FK significantly enhanced shoot (33.3%) and root lengths (44.5%), dry weights of plants (58.1%), chlorophyll a (72.7%), chlorophyll b (115.7%) and total chlorophyll content (88.4%) in spinach under drought. More spinach growth, under drought conditions, subjected to SA600 and FK was linked with better root elongation and nutrients (N, P and K) uptake. In conclusion, co-application of SA and K has more potential to improve spinach growth under drought stress compared to their sole applications. However, SA600 and FK are more effective levels compared to SA300 and HK for the alleviation of native effects of drought in spinach.

Key words: Drought, Growth attributes, Potassium, Salicylic acid, Spinach.

Introduction

Spinach (*Spinacia oleracea* L.), is one of the most famous leafy vegetable crops with minimum growth cycle and is an annual plant, associated to family *Chenopodiaceae* (Biemond *et al*., 1996). Spinach was first cultivated in North Africa. After that is came from Spain to North Europe (Dicoteau, 2000; Swiader & Ware, 2000). Spinach has a high nutritive value due to the presence of important minerals and vitamins. Among nutrients, spinach is a good source of calcium, vitamin C, phosphorous, iron, potassium and sodium (Dicoteau, 2000). It is a highly loveable leafy vegetable with a good cooking adoptability (Nishihara *et al*., 2001). The United States, China, Japan, Indonesia and Turkey are among the largest commercial producers of spinach (Anon., 2009). In China, 2768 kg ha^{-1}, United States 2360 kg ha^{-1}, Japan 12471 kg ha^{-1}, Indonesia 3424 kg ha^{-1} and Turkey 9249 kg ha^{-1} are average productions of spinach (Anon., 2009). However, with the passage of time, the changes in climate has decreased the availability of irrigation water for the cultivation of crops which is resulting in the development of drought stress (Anjum *et al*., 2011).

Drought is considered the most crucial among all abiotic stresses that limit the productivity of crops (Anjum *et al*., 2011; Danish *et al*., 2014; Danish and Zafar-ul-Hye *et al*., 2019). Every year, out of 75% useable irrigational water, only 10 to 30% is consumed while rest is wasted (Yu *et al*., 2013). High temperature and low level of precipitation also favoured the establishment of drought condition (Szilagyi, 2003). It is well documented that drought stress annually decreases the yield of crops up to 50%. Such a reduction in the yield of crops is mainly attributed to a disturbance in the biochemical and physiological process under drought condition (Hoekstra *et al*., 2001). Many scientists also noted that under the drought stress, plants usually produce and accumulate the higher amount of ethylene (Mayak *et al*., 2004; Zahir *et al*., 2008; Zafar-ul-Hye *et al*., 2019) which results in reduced plant yield.

The stress induced by ethylene plays an important role in evoking the physiological responses under drought (Wang *et al*., 2003). To overcome the problem of drought most of the scientists recommended to apply salicylic acid (SA) that takes part in stress signalling against several abiotic as well as biotic stresses (Ashraf *et al*., 2013). Foliar application of SA has been found effective in mitigation of stress (high temperatures and drought) tolerance in plants (Wang and Li, 2006). Salicylic acid is involved in ion transport regulation, modulating stomatal opening and detoxifying harmful effects of heavy metals (Rai, 2002). The osmotic stress caused by drought and salinity stress is alleviated to a greater proportion by exogenous application of SA (Borsani *et al*., 2001). The exogenous application of SA has been proved to be useful in enhancing the yield of crops including wheat (Shakirova *et al.,* 2003), rice (Tamaoki *et al*., 2013), barley (El-Tayeb, 2005), sunflower (Noreen *et al*., 2009) and cotton (Noreen *et al*., 2013).

Al-Hakimi & Hamada, (2001) observed that the application of SA at the rate of 100 mg L^{-1} proved highly effective, in alleviating the negative impact of salinity stress on wheat seedlings. The internal quantum and built up osmolytes via various exogenous applications of growth regulators and hormones result in enhancing the osmotic adjustment by scavenging free radicals (Ali & Ashraf, 2011). On the other hand role of better availability of potassium is also established that regulate stomatal conductance via osmoregulation mechanism under drought stress conditions (Wilkinson & Davies, 2002; Shabala, 2003). So, the current study was conducted with the aim to explore the combined effect of salicylic acid and various levels of potassium on growth and chlorophyll content of spinach under drought stress. It is hypothesized that combined application of salicyclic acid and recommended application of potassium is a better approach to mitigate drought stress in spinach.

Materials and Methods

Soil collection and characterization: Soil was collected from a cultivated area of agricultural farm near old Shujabad road Multan, Punjab, Pakistan. For pre-experiment soil characterization, hydrometer method was used for determination of soil separates (Sand = 60%, Silt = 30% and clay = 10%). USDA textural triangle was used for textural class which was sandy loam (Gee and Bauder, 1986). For assessment of soil organic matter (0.60%) Walkley (1935) protocol was followed. Olsen method was used for soil extractable phosphorus (7.58 mg kg^{-1}) (Olsen and Sommers, 1982), while Nadeem *et al*., (2013) methodology was adopted for determination of extractable potassium (185 mg kg^{-1}).

Pots dimension and preparation: Polythene bags were used as pots having dimensions 15 cm diameter and 30 cm depth. In each bag, 5 kg soil was filled. To fulfill nutrients requirement, nitrogen (N) and phosphorus (P) were applied at the rate of 22 and 15.6 kg ha^{-1}. For application of potassium (K) treatment plant was followed. To achieve half dozen of recommended potassium fertilizer (HK), 13.8 kg ha^{-1} while for full doze of recommended potassium fertilizer (FK) 27.6 kg ha^{-1} K_2O was applied as suggested by production technology of Anon., (2018).

Drought stress: Drought stress was maintained through field capacity (35%). Initially, 1 kg of soil was taken and a sufficient amount of water was added to make it fully saturated. After that pot was left for 24h so that gravitational water may leach out through gravitational pull. Finally, 100 % field capacity (FC) was calculated by using the equation.

$$\text{FC (\%)} = \text{Water added} - \text{Water leached}$$

However, during experiment pots were weighed at the time of irrigation to maintain the desired soil water level (35% FC) by adding an appropriate amount of water.

Treatments plan and experimental design: There were three levels of K and SA with three replications arranged in two factorials completely randomized design (CRD). The treatments were: control (No SA + No K), SA300 (300 µM), SA600 (600 µM), HK (13.8 kg ha^{-1} = 34.5 mg / 5 kg soil), FK (27.6 kg ha^{-1} = 69.0 mg / 5kg), SA300 + HK, SA600 + HF, SA300 + FK and SA600 + FK.

Seeds collection and sowing: Certified seeds of Desi Palak (*Spinacia oleracea*) was collected from the local market. Initially all weak and damaged seeds were manually screened out. After that in each pot 10 seeds of Desi Palak were sown. When seeds become germinated 5 seedlings were maintained in each pot by thinning.

Time and application rate of salicylic acid: There were three levels of salicylic acid (SA) i.e., control (no salicylic acid), 300 µM SA and 600 µM SA. For foliar application, salicylic acid was dissolved in ethanol and 0.01% Tween-20 was also added for penetration purpose and the desired volume was made by using deionized water. Both levels of SA (300 µM and 600 µM) were sprayed on 21st and 35th days after germination.

Harvesting: Spinach plants were harvested after 60 days of sowing. Shoot and root lengths were measured by using measuring tape. Fresh plant weight was noted on digital weight balance. Samples were oven dried at 60°C for 72 h as described by Zafar-ul-Hye *et al*., (2018).

Chlorophyll and accessory pigments: For examining the chlorophyll a, chlorophyll b and total chlorophyll in the leaves, 0.1g fresh leaf was cut from the shoot. Crushing was performed in a mortar by adding 5ml of 80% acetone solution. Final volume of 10 mL was made by using acetone (80%). At the end, filtration was done by using Whatman No.42 filter paper. For determination of absorbance at 645 and 663 nm wavelength spectrophotometer was used. The final chlorophyll a, chlorophyll b and total chlorophyll contents in spinach leaves was evaluated by using the formulae of Arnon, (1949):

$$\text{Chlorophyll a (mg g}^{-1}\text{)} = \frac{12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645) \times \text{V}}{1000\ (\text{W})}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)} = \frac{22.9\ (\text{OD } 645) - 4.68\ (\text{OD } 663) \times \text{V}}{1000\ (\text{W})}$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

For calculation of carotenoids, the following anthocyanin and lycopene equations were used (Kirk and Allen, 1965; Rao *et al*., 1998; Sims *et al*., 2002).

$$\text{Carotenoids (mg g}^{-1}) = \text{OD480} + 0.114\ (\text{OD 663}) - 0.638\ (\text{OD 645})$$

$$\text{Anthocyanin } (\mu\text{mol ml}^{-1}) = (0.08173 \times \text{OD 537}) - (0.00697 \times \text{OD 645}) - (0.002228 \times \text{OD 663})$$

$$\text{Lycopene } (\mu\text{g g}^{-1}) = \frac{(\text{OD 503} - 0.0007) \times 30.2}{\text{g plant tissue}}$$

where,
OD = Optical density (wavelength)
V = Final volume made
W = Fresh leaf weight (g)

Electrolyte leakage: Leaf discs of equal size (0.3g) were dipped in 15ml of deionized water (DI) water and incubated for 2 hours at 25ºC in test tubes. Initial EC of the solution (EC1) was taken after incubation. Samples were again autoclaved at 120°C for 20 minutes and final EC (EC2) was measured after equilibrium at 25°C. Electrolyte leakage will be measured using the method Lutts *et al*., (1996).

$$\text{EL } (\%) = \frac{\text{EC1}}{\text{EC2}} \times 100$$

Statistical analysis

All the statistical analysis was performed by following standard statistical techniques (Steel *et al*., 1997). Data were analyzed by following two factorials analysis of variance (ANOVA). Tukey's test was applied at $p \leq 0.05$ for comparison of treatments. For statistical analyses statistical computer software package SPSS was used (SPSS Inc. released 2009. PASW Statistics for Windows, Version 18.0 Chicago).

Results

Shoot and root length: Both main and interactive effects of various levels of potassium application rates (K) and salicylic acid (SA) were significant ($p \leq 0.05$) for shoot and root length of spinach under drought stress. Application of SA600 along with HK and FK performed significantly ($p \leq 0.05$) better over control (No K + No SA) for shoot length under drought stress (Fig. 1). Likewise, SA600, SA300 + HK, FK and SA300 + FK remained statistically similar to each other but significantly ($p \leq 0.05$) different from control (No K + No SA) for shoot length (Fig. 1). No significant change was noted where HK and FK were applied without SA. However, both HK and FK also remained significantly ($p \leq 0.0.05$) better from control for shoot length of spinach under drought stress. Maximum increases of 149.6, 36.3, 33.3% in shoot lengths were noted where no SA, SA300 and SA600 were applied at control (No K + No SA), HK and FK respectively under drought stress. In case of root length, SA600 + FK and SA600 + HK did not differ significantly ($p \leq 0.05$) with each other but remained significantly ($p \leq 0.05$) better from control (No K + No SA) under drought stress. Likewise, SA300 + HK, FK and SA300 + FK remained statistically similar to each other but differed significantly ($p \leq 0.05$) from control (No K + No SA) for root length of spinach under drought (Fig. 1). It was observed that without SA, FK was significantly ($p \leq 0.05$) different from control (0K) while HK and control did not differ significantly ($p \leq 0.05$) from control for spinach root length under drought. Maximum increases of 87.3 and 44.5% in root lengths were noted where SA300 and SA600 were applied at HK and FK respectively under drought stress.

Plant fresh and dry weight: Both main and interactive effects of K and SA were significantly ($p \leq 0.05$) different for plant fresh and dry weight under drought stress. Statistical analysis confirmed that SA600 + FK, SA300 + FK and SA600 + HK remained statistically similar to each other but performed significantly ($p \leq 0.05$) better from control (No K + No SA) for plant fresh weight under drought stress. Application of FK was significantly ($p \leq 0.05$) different while HK did not differ significantly ($p \leq 0.05$) over control (0K) for plant fresh weight (Fig. 2). Both SA300 and SA600 also remained significantly ($p \leq 0.05$) better from control for plant fresh weight under drought stress. Maximum increases of 136.8, 74.5 and 48.2% in plant fresh weighs were noted where No SA, SA300 and SA600 were applied at control (0K), HK and FK respectively under drought stress. For plant dry weight, SA600 + FK and SA300 + FK remained statistically alike to each other but differed significantly ($p \leq 0.05$) better from control (No K + No SA). It was observed that HK, SA300 + HK and SA600 + HK did not differ with each other for plant dry weight under drought stress (Fig. 2). However, application of SA600 performed significantly ($p \leq 0.05$) better but S30 remained statistically alike from control (No K + No SA) for plant dry weight. Maximum increases of 57.3% and 58.1% in plant dry weighs were observed where No SA and SA600 were applied at control (0K) and FK respectively under drought stress.

Chlorophyll content: Both main and interactive effects of K and SA were significantly ($p \leq 0.05$) different for chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) under drought stress. It was observed that SA600 + FK, SA300 + FK, SA600 + HK and SA300 + HK remained statistically similar to each other but differed significantly ($p \leq 0.05$) better over control (No K + No SA) for chlorophyll a content. No significant change was noted where sole application of FK, HK, SA600 and SA300 were done from control (No K + No SA) for chlorophyll a content in spinach under drought stress (Fig. 3). A maximum increase of 72.7% in chlorophyll a content was noted from control (No K + No SA) where SA600 + FK was applied. For chlorophyll b, SA600 + FK, SA300 + FK, SA600 + HK and SA300 + HK performed significantly ($p \leq 0.05$) better from control (No K + No SA) under drought stress. Application of FK, HK without SA did not differ significantly ($p \leq 0.05$) from control (No K + No SA) for chlorophyll b content in spinach under drought stress (Fig. 3). Similarly, sole application of SA300 and SA600 also remained statistically alike with control (No K + No SA) for chlorophyll b content. A maximum increase in chlorophyll b i.e., 115.7% was observed in SA600 + FK from control (No K + No SA) under drought stress. In case of total chlorophyll content in spinach, SA600 + FK, SA300 + FK, SA600 + HK and SA300 + HK differed significantly ($p \leq 0.05$) better from control (No K + No SA) under drought stress. Sole application of FK performed significantly ($p \leq 0.05$) better from HK and control (No K + No SA) for total chlorophyll content under drought stress (Fig. 3). In addition, SA600 and SA300 did not differ significantly ($p \leq 0.05$) for total chlorophyll content from control (No K + No SA). A maximum increase of 88.4% in total chlorophyll content was noted where SA600 + FK was applied over control (No K + No SA) under drought stress.

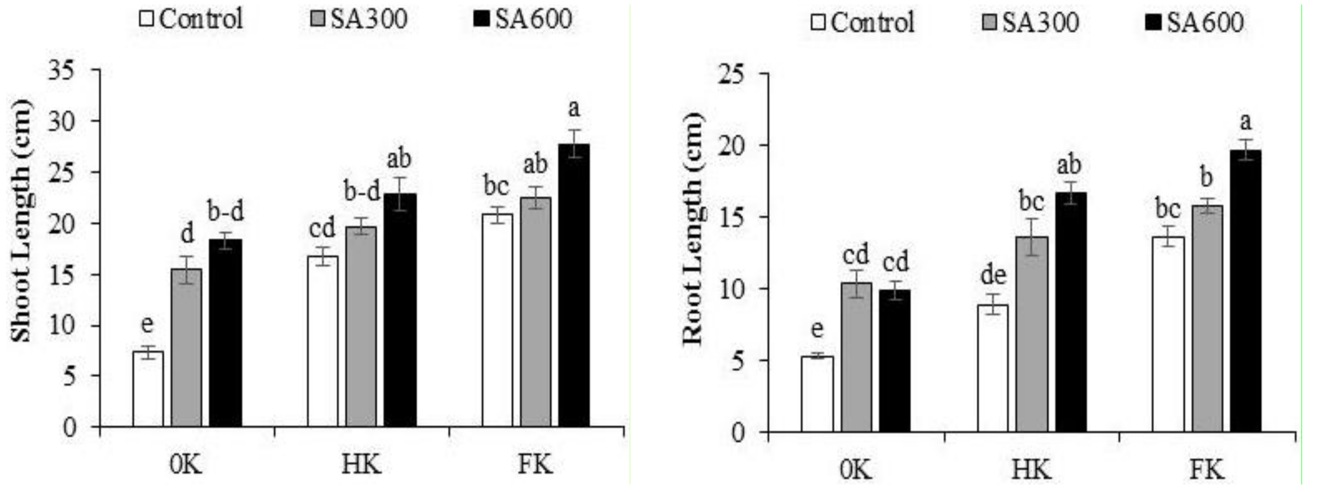

Fig. 1. Effect of various levels of salicylic acid (SA) and potassium (K) on shoot and root length (cm) of spinach under drought stress.

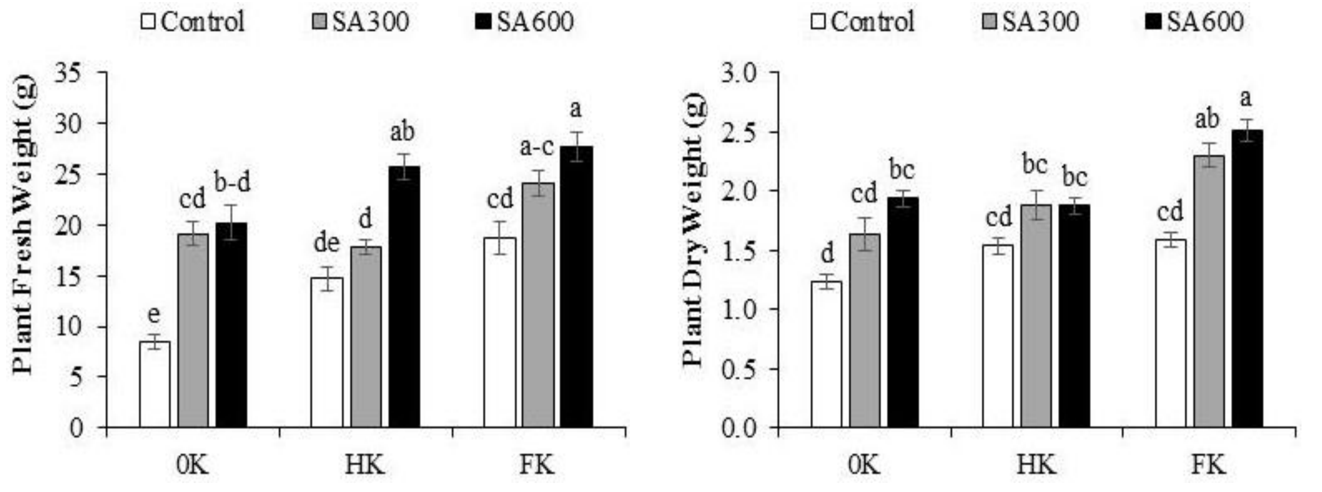

Fig. 2. Effect of various levels of salicylic acid (SA) and potassium (K) on plant fresh and dry weight (g) of spinach under drought stress.

Accessory pigments: Both main and interactive effects of K and SA were significantly ($p \leq 0.05$) different for accessory pigments (carotenoids, anthocyanin and lycopene) under drought stress. Application of SA600 + FK, SA300 + FK, FK and SA300 + HK performed significantly ($p \leq 0.05$) better from control (No K + No SA) for carotenoids in spinach leaves under drought stress. Both HK and FK without SA remained statistically alike to each other but differed significantly ($p \leq 0.05$) better from control (No K + No SA) for carotenoids in spinach leaves under drought stress (Fig. 4). It was noted that SA300 + HK was statistically similar to SA600 and SA300 for carotenoids under drought. However, sole application of SA600 and SA300 performed significantly ($p \leq 0.05$) better over control (No K + No SA) for carotenoids under drought stress. A maximum increase of 158.3% in carotenoids was noted in SA600 + FK from control (No K + No SA). For anthocyanin, application of SA600 + FK and SA300 + FK remained significantly ($p \leq 0.05$) better over control (No K + No SA) under drought stress. It was observed that application of SA300 + FK, SA300 + HK were statistically similar to each other but remained significantly ($p \leq 0.05$) different from sole application of SA300 and control (No K + No SA) for anthocyanin in spinach leaves under drought stress (Fig. 4). No significant change was noted in anthocyanin where sole application of HK and

FK was done from SA300 and control (No K + No SA) under drought. However, sole application of SA600 remained significantly ($p≤0.05$) better from SA300 and control (No K + No SA) for anthocyanin under drought. A maximum increase of 142.9% in anthocyanin was noted where SA600 + FK was applied from control (No K + No SA) under drought stress. Application of SA600 + FK, SA300 + FK, SA600 + HK significantly ($p≤0.05$) decreased lycopene over control (No K + No SA) under drought stress (Fig. 4). It was observed that sole application of SA300, SA600, HK, SA300 + HK and FK remained statistically alike to each other but significantly ($p≤0.05$) decreased lycopene in spinach from control under drought. Maximum reduction of 61.7% in lycopene was observed from control (No K + No SA) where SA600 + FK were applied.

Shoot N, P and K concentration: Main effects of SA and K were significant but their interaction remained non-significant for N, P and K concentration in spinach leaves. Application of SA600 and FK remained significantly ($p≤0.05$) better over control for N and P concentration in spinach leaves under drought stress (Tables 1-2). Similarly, SA300 and HK also performed significantly ($p≤0.05$) better from control for N and P concentration in spinach leaves under drought stress. The maximum increases of 44.6% and 44.3% in N and P concentration in leaves were noted over control (No SA) where SA600 was applied. Similarly, application of FK gave maximum increase of 33.1 and 33.2% in N and P concentration in spinach leaves from control (No K). In the case of K concentration in spinach leaves, no significant change was noted where HK and FK were applied. However, both HK and FK remained significantly ($p≤0.05$) better from control (0K) for K concentration in spinach leaves under drought stress (Table 3). Application of SA600 remained significantly ($p≤0.05$) better from SA300 and control (No SA) under drought stress. Likewise, SA300 also performed significantly ($p≤0.05$) better for K concentration in spinach leaves over control (0 SA) under drought stress. The maximum increase of 103.5 and 15.3% in K concentration in spinach leaves were noted where SA600 and FK were applied from control (0 K + 0 SA).

Electrolyte leakage: Main effect of SA and K were significantly ($p≤0.05$) different but interactive effects of SA and K remained non-significant for electrolyte leakage in spinach leaves under drought. Application of SA600 significantly ($p≤0.05$) decreased electrolyte leakage in spinach leaves from SA300 and control. Likewise, SA300 also performed significantly ($p≤0.05$) better from control for reduction in electrolyte leakage in spinach leaves under drought (Table 4). On the other hand, the increasing application rate of K also significantly ($p≤0.05$) decreased electrolyte leakage in spinach leaves from control in spinach leaves under drought. Maximum reduction in electrolyte leakage were noted in SA600 (35.4%) and FK (43.6%) from respective controls in spinach under drought stress.

Table 1. Effect of various levels of salicylic acid (SA) and potassium (K) on N concentration (%) in spinach leaves under drought stress

Levels of salicylic acid (SA)	Shoot nitrogen (%)			
	Various application rate of potassium			
	IE (SA × K)			ME (K)
	No potassium (0K)	Half potassium (HK)	Full potassium (FK)	
Control (No SA)	1.69	1.83	2.27	1.93 C
300 µM SA (SA30)	1.75	2.29	2.71	2.25 B
600 µM SA (SA60)	2.14	2.48	3.09	2.57 A
ME (SA)	1.86 C	2.20 B	2.69 A	

Mean are average of 3 replicates. Different letters on main effects showed significant difference at $p≤0.05$
ME = Main effect; IE = Interactive effect

Table 2. Effect of various levels of salicylic acid (SA) and potassium (K) on P concentration (%) in spinach leaves under drought stress

Levels of salicylic acid (SA)	Shoot phosphorus (%)			
	Various application rate of potassium			
	IE (SA × K)			ME (K)
	No potassium (0K)	Half potassium (HK)	Full potassium (FK)	
Control (No SA)	0.16	0.18	0.23	0.19 C
300 µM SA (SA30)	0.17	0.23	0.27	0.22 B
600 µM SA (SA60)	0.22	0.24	0.30	0.25 A
ME (SA)	0.18 C	0.22 B	0.26 A	

Mean are average of 3 replicates. Different letters on main effects showed significant difference at $p≤0.05$. ME = Main effect; IE = Interactive effect

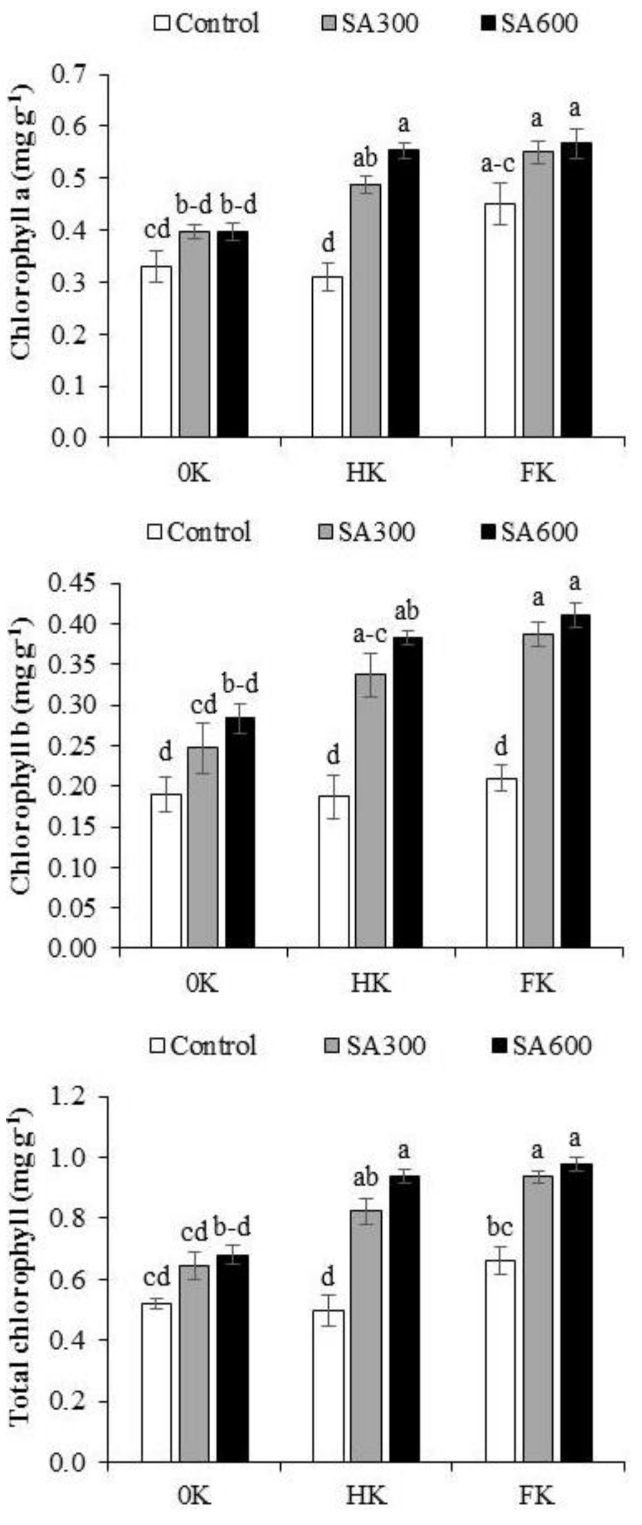

Fig. 3. Effect of various levels of salicylic acid (SA) and potassium (K) on chlorophyll a, chlorophyll b and total chlorophyll (mg g^{-1}) of spinach under drought stress.

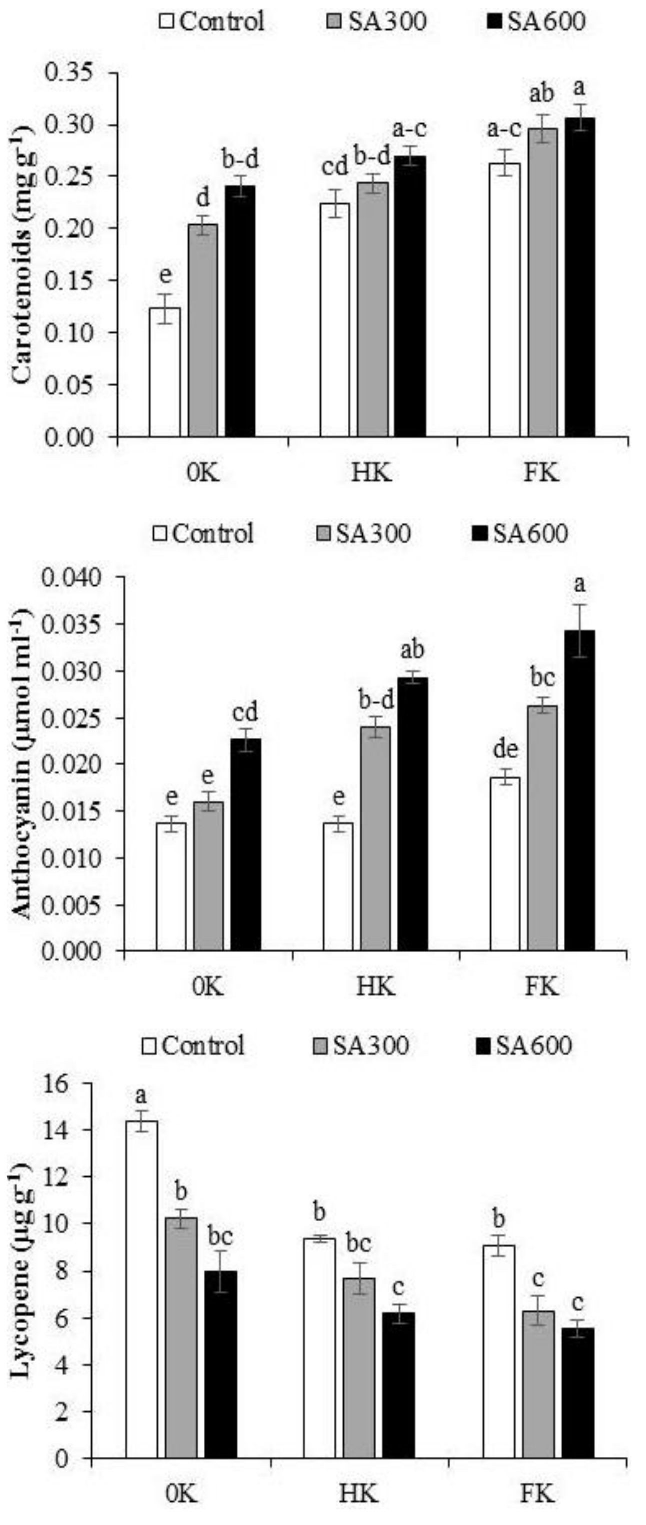

Fig. 4. Effect of various levels of salicylic acid (SA) and potassium (K) on carotenoids (mg g^{-1}), anthocyanin (µmol ml^{-1}) and lycopene (µg g^{-1}) of spinach under drought stress.

Discussion

In current pot study, drought stress substantially decreased growth attributes in control while application of SA and K especially SA600 + FK counteract adverse effects of drought. Impairment of cell division and elongation might be possible reasons for reduction in growth of root and shoot length in spinach under drought (Taiz & Zeiger, 2010). Baalbaki *et al*., (1999) and Hellal *et al*., (2018) also noticed similar kind of results regarding decrease in root and shoot elongation under drought stress. It is an established fact that less cell division and restriction of elongation are key factors responsible for reduction in root and shoot length as well as dry weights (Taiz & Zeiger, 2010; Hussain *et al*., 2018; Paul *et al*., 2018; Danish *et al*., 2019) as observed in current study.

According to Gargallo-Garriga *et al*., (2014), deactivation of metabolic processes under drought stress that decreases shoot length. Limited availability of NPK in current study was also linked with less root elongation and limited water uptake under drought stress. It is quite common that nutrients uptake is decreased in crop plants when they are subjected to drought stress as a result of impaired root system (Izzo *et al*., 1989; Hussain *et al*., 2018).

Likewise, a significant reduction in dry weight was linked with poor root growth, low nutrients uptake and reduction in chlorophyll contents. The reduction in chlorophyll content was possibly due to higher synthesis of ethylene (Matile *et al*., 1997). Drought stress increased the production of stress generating ethylene that deteriorates the integrity of cell membrane via lipid degradation. Due to lipid degradation ethylene become in direct contact with chloroplast and activates chlorophyllase (chlase) gene, resulted in severe damage of chlorophyll (Matile *et al*., 1997). Foliar application of SA300 and SA600 significantly increased growth attributes in spinach under drought stress possibility due to modulation of enzymatic activities that play an important role in mitigation of drought stress. The findings of Alam *et al*., (2013) also validated our argument as they noted modulation of various enzymes i.e., dehydroascorbate reductase, DHAR; monodehydroascorbate reductase, MDHAR; GR; GSH peroxidase, GPX) by exogenous application of SA. Saruhan *et al*., (2012) suggested that improvement in defense mechanism of plants by application of SA (1.0 μM) is another important feature due to which growth is improved under drought stress.

Nazar *et al*., (2015) argued that restriction of 1-aminocyclopropane carboxylic acid synthase (ACS) activity (ethylene synthesis) and higher synthesis of proline improve the photosynthesis in plants under drought. Low accumulation of ethylene by deactivation of 1-aminocyclopropane carboxylic acid synthase (ACS) activity was possibly linked with less electrolyte leakage by application of SA under drought stress. Noreen *et al*., (2011) suggested that application of SA maintained the turgor potential that is key factor responsible for improvement in chlorophyll a, b and total chlorophyll content which was also observed in current study. Likewise, an improvement in N uptake was also important factor for increase in chlorophyll content. However, the reduction in electrolyte leakage in current study via application of K might also be due to better uptake of K which maintained cell turgor. Raza & Saleem (2013) noted that balance uptake of K adjusts the osmotic potential of plants that significantly improved relative water content and nutrients uptake under drought stress. The improvement in stomatal conductance by foliar application of 1% K also enhanced the synthesis of carbohydrates under drought stress (Bahrami-Rad & Hajiboland, 2017).

Table 3. Effect of various levels of salicylic acid (SA) and potassium (K) on K concentration (%) in spinach leaves under drought stress.

Levels of salicylic acid (SA)	Shoot potassium (%)			
	Various application rate of potassium			
	IE (SA × K)			ME (K)
	No potassium (0K)	Half potassium (HK)	Full potassium (FK)	
Control (No SA)	1.17	2.21	2.71	2.03 B
30 mM SA (SA30)	1.49	2.47	2.82	2.26 A
60 mM SA (SA60)	1.53	2.54	2.95	2.34 A
ME (SA)	1.39 C	2.41 B	2.83 A	

Mean are average of 3 replicates. Different letters on main effects showed significant difference at $p \leq 0.05$. ME = Main effect; IE = Interactive effect

Table 4. Effect of various levels of salicylic acid (SA) and potassium (K) on electrolyte leakage (%) in spinach leaves under drought stress.

Levels of salicylic acid (SA)	Electrolyte leakage (%)			
	Various application rate of potassium			
	IE (SA × K)			ME (K)
	No potassium (0K)	Half potassium (HK)	Full potassium (FK)	
Control (No SA)	68.67	59.33	53.00	60.33 A
30 mM SA (SA30)	54.67	45.00	38.67	46.11 B
60 mM SA (SA60)	46.33	37.67	18.00	34.00 C
ME (SA)	56.56 A	47.33 B	36.56 C	

Mean are average of 3 replicates. Different letters on main effects showed significant difference at $p \leq 0.05$. ME = Main effect; IE = Interactive effect

Conclusion

Drought stress impaired spinach growth while salicylic acid i.e., SA600 and a full dose of recommended K (FK) can mitigate drought stress. The improvement in spinach growth, nutrients uptake and chlorophyll content with SA600 + FK were primarily linked with root elongation, better NPK uptake and less electrolyte leakage. However, more investigations are needed at field level to introduce SA600 + FK to improve the growth of *Spinacia oleracea* under drought stress.

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