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INFLUENCE OF SALICYLIC ACID AND POTASSIUM NITRATE ON PLANT HEIGHT AND FLOWERING TIME OF GROUNDNUT (*ARACHIS HYPOGAEA L.*) UNDER VARYING SALINITY AND DROUGHT- INDUCED STRESSES

P. Nkrumah, A. M. Amadu And K. O. Ayeh*
(Department of Plant and Environmental Biology, School of Biological Sciences, College of Basic and Applied Sciences, University of Ghana, Legon, Ghana).
**Corresponding author's email: koayeh@ug.edu.gh*

ABSTRACT
This study was conducted to examine the effects of Salicylic acid (SA) and Potassium nitrate (KNO_3) on plant height and flowering time of groundnut (*Arachis hypogaea* L.) under induced stresses of salinity, drought and combined salinity and drought. Three watering regimes namely; normal, moderate and severe were used. For salinity stress, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl and combination of water and salinity regimes were used. 50 µm KNO_3 and 50 µm SA were separately applied to plants under water, salinity and combined water and salinity stresses. The mean plant height at 100 mM salt concentration were 42.29 cm for KNO_3, 42.27 cm for SA, compared with 40.98 cm for control ($F = 2.73$; $P \leq 0.008$). In combined severe watering and 150 mM NaCl treatment, flowering time was 57 DAP (days after planting) compared to 34 DAP in control plants. When KNO_3 and SA were applied to severe watering and 150 mM NaCl combined stress induced plants, flowering time was 51 DAP and 53 DAP for KNO_3 and SA treated plants respectively. In conclusion, the exogenous application of plant growth regulators such as SA and KNO_3 reduce impact of water stress on groundnut and effectively improve yield.

Keywords: Water and salinity stress, *Arachis hypogaea* L., salicylic acid, potassium nitrate, plant height and flowering

Introduction

Arachis hypogaea L. (groundnut) is one of the world's most popular crops cultivated throughout the tropical and sub-tropical areas where annual precipitation is between 1000 - 1200 mm for optimum growth of the crop (Shiyam, 2010). Groundnuts have a high nutrient content comprising 44 - 56% oil, 22 - 30% protein, 9.5 - 19.0% carbohydrates and a rich source of minerals (Jung *et al*., 2003) including calcium, magnesium, phosphorus, and potassium and vitamins E, K, and B (Gupta, 2012). Nonetheless, the cultivation of groundnuts may be negatively impacted by abiotic stress factors such as drought and salinity stresses not excepting plant disease.

Drought stress has become the main environmental factor leading to reduced agricultural productivity and food safety worldwide and has, therefore, become a major concern in crop production (Bodner *et al*., 2015; Fahad *et al*., 2017). Plants tolerate water deficit stress by morpho-physiological, biochemical, and anatomical adaptive mechanisms that involve osmotic change and stomatal closure, thereby enabling plants to withstand water stress (Basu *et al*., 2016).

Salinity is a significant stress impacting worldwide crop productivity (Pitman & Läuchli, 2002). More than 20% of the world's cultivated land is affected by salt stress and the average volume of salt increases (Gupta & Huang, 2014). Salinity stress responses involve changes in different physiological and metabolic processes influenced by the intensity and length of the stress and eventually inhibits crop production (Pradheeban *et al*., 2017). During the initial phases of salinity stress, the water absorption potential of the root systems decreases, and water deficiency in leaves is increased by high salt and accumulation of other osmolytes in the soil (Hanumantharao *et al*., 2016; Gupta & Huang, 2014);

Growth in height is necessary in most plants as it helps plants access light needed for its general growth and survival (Nagashima & Hikosaka, 2011; Falster & Westoby, 2003). Plant height usually increase as temperatures drop and soil conditions become moistier (Olson *et al.*, 2018). This is evident in the vegetation zones in Ghana where herbs and shrubs are prominent in the savanna or desert areas and taller trees being prominent in the deciduous forest and rain forest zones (Asravor *et al*., 2019). When factors necessary to provide a plant with water needed to maintain turgor in its aerial tissues are limited, it results in water deficit or stress and plant height may be impacted (Niklas, 2007). Thus, diseases and other unfavourable ecophysiological conditions such as drought and salinity result in plant height reduction (Baher *et al*., 2002; Saadatmand *et al*., 2007).

Flowering is an important phase in the life cycle of angiosperms because it ensures seed dispersal through agents of pollination such as wind, insects, birds and bees. The process of flowering is dependent on prevailing environmental conditions and internal genetic factors so that there is transition from vegetative to reproductive growth and optimum conditions are met for fertilization and seed formation (Kazan & Lyons, 2016; Liang *et al*., 2019). Drought causes early arrest of floral development and leads to sterility (Su *et al*., 2013). Recent findings have shown that combined drought and heat stress during evocation may reduce silk and ovary growth, extend anthesis-silking interval in kernel number per plant in maize (Liu *et al*., 2020). Furthermore, there have been reports of the close association between flowering time and drought conditions in *Sarcopoterium spinosum* (Waitz *et al*., 2021). Studies have revealed that salt induced stress reduced flowering and fruit ripening, reduced fruit size and number per plant (Koffi *et al*., 2019). Recent research has indicated that plants ability to tolerate salinity stress do not only depend on species specificity but also on the degree of salinity tolerance experienced by the plant (Zunzunegui *et al*., 2021).

Plants have adapted to a broad array of mechanisms during their evolution to withstand a range of stressful conditions (Lamalakshmi Devi *et al*., 2017). These include the synthesis of potassium containing chemicals such as KNO_3 and plant growth regulators such as SA. There is significant evidence that mineral nutrients play a critical role in a plant's resistance to stress. Out of all the mineral nutrients, potassium (K) plays a critical role in plant growth and metabolism, and it contributes greatly to the survival of plants that are under various abiotic stresses. Potassium is an important mineral element in plants and is therefore considered the most abundant in most soils. It has been consistently found that the concentration of K^+ in the cytoplasm is between 100 and 200 mM and that the concentration of apoplastic K^+ may vary between 10 and 200 or even reach up to 500 mM (Wang *et al*., 2013).

Salicylic acid (SA; 2-hydroxybenzoic acid) is one of several plant-synthesized phenolic compounds containing a benzene ring that carries one or more hydroxyl groups (Klessig & Malamy, 1994). SA is an essential plant hormone that regulates plant growth and development (Dempsey & Klessig, 2017). SA has been shown to enhance plant tolerance to major abiotic stresses such as salinity, drought, toxic metals and heat (Hasanuzzaman *et al*., 2017; Khan et al. 2015). It has been documented that exogenous application of SA to stressed plants either by seed soaking, addition to nutrient solution, irrigation or spraying induced significant biotic stress tolerance in plants (El-Katony *et al*., 2019; Khan *et al*., 2015). Information on combined abiotic stresses on plants such as groundnuts and some ameliorating effects to counteract some of the negative morphological and physiological responses of plants are scanty. The objective of this study, therefore, was to investigate the effects of varying water deficits, salinity and their combination on growth of economically important crop such as groundnut plant and integrate stress responses with flowering time and plant height. The influence of the mitigating effects of SA and KNO_3 on plant height and flowering time of groundnut under water and salinity stresses were also investigated.

Experimental

Plant material

The study was conducted using a commercially grown groundnut variety in Ghana, Kumawu Red.

Soil

The soil type used in this study was Haatso – Nyibgenya Series, obtained from the teaching garden in the Department of Plant and Environmental Biology, University of Ghana and sterilized at a temperature of 121^{o} C and a pressure of 15 psi for 20 mins. 30 g of soil was weighed and placed into polythene bags of dimension 20 x 30cm. A total of 105 bags were used for the study.

Study site

Seedlings were placed on a cement platform in the Screenhouse of the Department of Plant and Environmental Biology, University of Ghana at temperature of 33^{o}C and 75% relative humidity.

Experimental design

The experiment was carried out in Randomized Complete Block Design (RCBD) with split plot arrangement. It consisted of 5 replicates each with seven treatment blocks namely control, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, normal, moderate, severe, and severe watering and salt (150 mM NaCl) with each block having three different treatments (control, 100 mM SA and 100 mM KNO_3).

Watering regime

100 ml of water was used in watering each plant and all seedlings were saturated with water for the first four weeks under each treatment block. Three watering regimes were used in the study, namely, normal (rehydrated every other day), moderate (rehydrated every 3 days) and severe (rehydrated every 5 days).

NaCl solution preparation.

Commercially sold halite, commonly known as rock salt was used. Concentrations of 50 mM, 100 mM, and 150 mM were prepared. These were applied to plants that were subjected to the different concentrations of NaCl using a measuring cylinder.

Hormone application
20 ml of 50 µM SA solution was applied to plants, subjected to this treatment, using a measuring cylinder. This was done once a week for seven weeks.

Potassium nitrate application
Twenty (20) ml of 50 µM KNO_3 solution was applied to plant subjected to this treatment once a week.

Data collection
The height of each plant in all blocks was measured with a meter rule for seven (7) weeks. This was done by measuring from the surface of the soil to the terminal bud at the apex of the plant. Days to first anthesis were recorded for each test plant.

Data analysis
All data collected were statistically analysed using ANOVA from Minitab version 17 (Minitab, LLC).

Results

Effect of SA and KNO_3 on mean plant height treated with varying concentrations of NaCl
It was observed that, as NaCl concentration increased, there was a reduction in plant height (Table 1). However, plants treated with SA and KNO_3 under different NaCl concentrations recorded marginally higher mean plant height values compared to their respective controls treated with only NaCl (Table 1) although the differences were not statistically significant ($F = 2.73$, $P = 0.008$).

TABLE 1
Effect of KNO_3 and SA on plant height under varying concentrations of NaCl.

	Mean plant height ± SE (cm)		
NaCl Conc. (mM)	Control ± SE	SA ± SE	KNO_3 ± SE
0	45.35±3.73ab	46.34±3.74ab	50.62±5.52a
50	41.55±1.45ab	45.55±3.04ab	47.87±6.62ab
100	40.98± 3.31ab	42.27±2.92ab	47.29±2.4ab
150	39.18±6.62b	42.17±5.75ab	42.88±3.14ab

Means that do not share a letter are significantly different.

Plants under NaCl stress treated with KNO_3 showed higher plant height values compared to their respective controls (Table 1) and they were significantly different ($F = 5.58$, $P = 0.000$).

Effect of SA and KNO_3 on mean plant height in plants treated with varying watering regimes at maturity
Plant height generally decreased as the water stress level increased, thus, plant height was inversely proportional to the increasing water stress. Plants treated with KNO_3 recorded

higher plant height compared to those treated with SA (Fig 1). Seedlings treated with SA recorded higher plant height values than the control plants (Fig. 1). The differences observed in plant height were statistically significant (F = 6.74, P = 0.00).

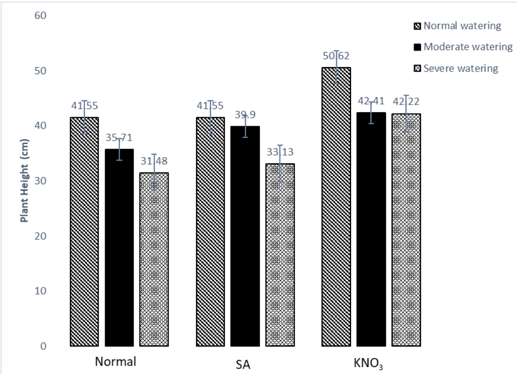

Fig. 1**:** Effect of SA and KNO_3 on plant height under different water stress (control, moderate and severe).

When plants treated with SA and KNO_3 under different watering regimes were compared, it was observed that plant height increased in both moderate and severe water stress levels (Fig. 2B & Fig. 2C) whereas control plants without SA and KNO_3 recorded reduced plant height (Fig. 2A).

Fig. 2: Effect of KNO_3 and SA on groundnut plant height under moderate watering 63 DAP. **A.** Control. **B.** Plants treated with SA. **C.** Plants treated with KNO_3.

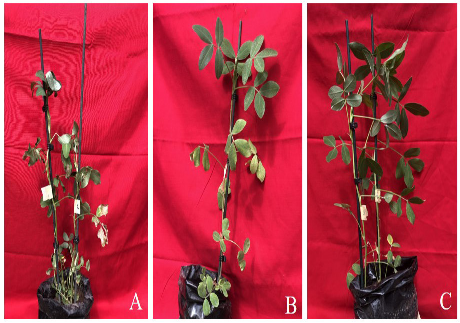

Fig. 3: Effect of SA and KNO_3 on plant height under severe water stress combined with high salinity stress of 150 mM NaCl at 63 DAP. **A.** Control. **B.** Plants treated with SA. **C.** Plants treated with KNO_3.

Combined high salinity concentration and severe water treatments

A comparison made between combined 150 mM NaCl and severe water treatment at 63 DAP showed that mean plant height decreased significantly (F =13.50, P = 0.00) in plants that were exposed to simultaneous water and salinity stresses (Fig. 3A) in contrast with the plants treated with KNO_3 and SA (Fig. 3B & 3C). It was also observed that plants that were treated with KNO_3 recorded higher plant heights followed by those that were treated with SA with control plants recording the lowest plant height of 33.8 cm (Fig. 4)

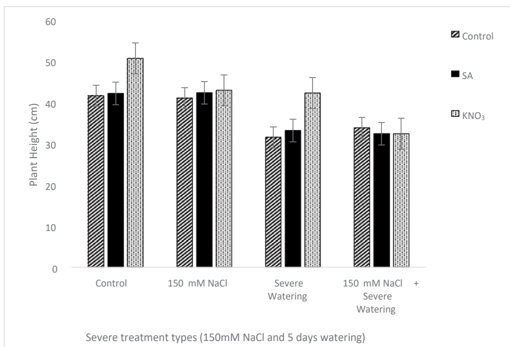

Fig. 4**:** Effect of SA and KNO_3 on severe treatment types.

Days to flowering (*Anthesis*)
First flowering was observed after 31 days. Plants that were not subjected to any stress flowered earlier compared to those that underwent stress (Table 2). It was also observed that plants under all treatment blocks treated with KNO_3 flowered earlier (32 DAP) compared to plants treated with SA whilst the control plants had extended flowering time by 2 to 7 days. The number of days to flowering were significantly different (F = 73.37, P = 0.00).

TABLE 2
Days to flowering in groundnut plants under all indicated treatments.

	Control ± SE	SA ± SE	KNO_3 ± SE
Normal (control)	34 ± 0.9ijk	33 ± 1.1k	32 ± 1.1k
50 mM NaCl	40 ± 2.41efgh	38 ± 2.9ghi	35 ± 1.140ijk
100 mM NaCl	42 ± 2.41ef	41 ± 2.42ef	40 ± 1.8fgh
150 mM NaCl	44 ± 1.8def	42 ± 1.1ef	41 ± 2.3efg
Moderate watering	37 ± 1.6hij	34 ± 1.1jk	32 ± 2.2ef
Severe watering	47 ± 1.6cd	44 ± 1.3de	43 ± 1.5k
Severe watering and 150 mM NaCl	57 ± 1.6a	53 ± 2.4b	51 ± 2.2bc

Means that do not share a letter are significantly different.

Discussion

Effect of NaCl stress on plant height
Groundnut plants exposed to NaCl stress exhibited a decrease in height as salinity stress is known to cause stunted growth in plants (Isayenkov, 2019). In this paper, plants that underwent severe NaCl stress recorded the least plant height compared to the others. Our observations in this paper agree with the findings of Acosta-Motos *et al*. (2017), who reported that water used for cell division and growth is moved out of the plant as a result of an increase in NaCl concentration in the soil.

Effect of SA and KNO_3 on plant height under NaCl stress
Both SA and KNO_3 improved plant height as compared to their controls. SA was able to ameliorate the negative effects of NaCl stress because it has been shown to reduce the damaging effect of salinity on plant growth, accelerate the restoration of growth processes and reverse the effects of salinity (Fayez & Bazaid, 2014). Potassium nitrate has been shown to be effective in mitigating the negative effects of salinity on coriander plants (Elhindi *et al*., 2016). This present study showed that NaCl stressed plants treated with KNO_3 had higher plant heights. This agrees with work by Ahmad *et al* (2019) who reported that KNO_3 increased plant growth by enhancing its physiological process as potassium (K) plays a key role in stomatal conductance, photosynthesis osmoregulation, protein synthesis and turgor pressure-driven solute transport in the xylem to plant.

Effect of water stress on plant height
Drought is known to cause stunted growth in plants (Yadav *et al*., 2020). Plants under water stress in this paper recorded a decrease in height compared to their controls. This agrees with results by Vurayai *et al*. (2011), which showed that water stress at different stages of growth and development of Bambara groundnut plants significantly reduced plant height compared to non-stressed control plants.

Effect of SA and KNO_3 on plant height under water stress

Results from this paper demonstrates that salicylic acid improved or increased the height of plants exposed to water stress. These findings agree with results of Nakandala *et al*. (2016) who reported that the application of SA to plants generally had a positive effect on vegetative growth parameters such as plant height and leaf size. Furthermore, Fayez & Bazaid (2014) also found that exogenous SA application was very effective in reducing the adverse effects of drought stress in sunflower**.** We also found that**,** higher plant height values were recorded in plants that were treated with KNO_3 as compared to those that were not treated with KNO_3 even though they were all undergoing drought stress. Wang *et al.* (2013) have reported that KNO_3 is known to promote cell elongation during drought stress in plants.

Combined effect of high salinity stress and severe water treatments on plant height

Drought and salinity stresses are known to have a profound negative effect on plants (Jaleel *et al*., 2009). Results from this present study showed that height of plants subjected to the combined effect of severe conditions of drought and saline stress recorded the least plant height values as observed by de Oliveira et al., (2016) who stated that drought and salinity stress are indeed known to reduce plant growth.

Effect of SA and KNO_3 on plant height under combined drought and high salinity stress.

SA has been shown to improve plant growth during water and salinity stress (Faghih *et al*., 2019; Nazar *et al*., 2015) not excepting an increment in plant height. Results from our investigations showed that there was an increase in height of plants exposed simultaneously to severe water and 150 mM NaCl when SA was applied. Studies from Manzoor *et al*., (2015) showed that SA at a concentration of 0.5 mM is more effective in contributing to the reduction of extreme saline impacts, significant increase germination rate and drought tolerance in maize seedlings. This study revealed that KNO_3 aided in reversing the negative effect of both severe drought and 150 mM NaCl stress on plant height. Fayez & Bazaid (2014) also showed that KNO_3 reversed the negative effects of drought and salinity stress in barley.

Effect of NaCl stress on flowering

In this paper, we reported that NaCl stress affected flowering time. Thus, plants exposed to higher concentration of NaCl recorded a delay in flowering compared to those exposed to lower concentrations of NaCl (Table 2). This agrees with the report by Julien *et al.* (2019) who stated that increasing salinity delayed plant flowering in a local cultivar of chili pepper (*Capsicum frutescens* L.).

Effect of SA and KNO_3 on flowering under NaCl stress

In our investigations, plants under NaCl stress treated with SA and KNO_3 flowered earlier as compared to their respective controls. However, it was further seen that plants under all three NaCl concentrations treated with KNO_3 flowered earlier than those treated with SA (Table 2). Flowering delayed as NaCl stress increased even though KNO_3 and SA were applied. These findings agree with that of Attia (2016) who indicated that under salt stress conditions, the application of SA and KNO_3 separately or combined accelerated flowering of olive trees.

Effect of water stress on flowering

Varying drought stress conditions may have different effects on flowering. Drought causes floral development to be arrested early and leads to sterility (Smith & Zhao, 2016).

Results from the paper of Kazan & Lyons (2016) showed that in *Arabidopsis,* drought stress accelerated flowering under long days but delayed flowering under short days. On the contrary, plants under severe water conditions delayed the most in flowering compared to plants subjected to moderate and normal watering in this present study similar to flowering of Arabidopsis under short days.

Effect of SA and KNO_3 on flowering under water stress

SA has been shown to reverse the effect of water stress on flowering (Hussain *et al*., 2008). In this study, it was observed that plants treated with SA under severe water stress conditions flowered earlier than their respective controls. However, flowering was delayed as stress level increased. Thus, plants under moderate watering regimes flowered earlier than those under severe watering regimes even though they were all treated with SA. These findings were similar to those of Rihan (2017) who showed that SA application enhanced the activities of different physiological and biochemical features, such as photosynthetic reactions, flowering, and plant growth which may also contribute to fruit yield and productivity in the wheat plant.

Conclusion

Plants treated with SA and KNO_3 reversed reduction of plant height in salt and water stressed plants. Delayed anthesis was reversed by application SA and KNO_3. However, ameliorating effect of KNO_3 was more pronounced as compared with SA in water and salinity induced stress treatments.

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