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Germination characteristics of *Sorghum bicolor* (L.) Moench under different pH regimes after chemopriming.

Características de germinación de *Sorghum bicolor* (L.) Moench bajo diferentes regímenes de pH después de quimiocebado

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ABSTRACT

Sorghum (*Sorghum bicolor* L.) Moench is an important crop that is predominantly produced in the Northern part of Nigeria. However, the challenge of desertification has consistently affected the soil pH, hindering the growth and yield of the crop. Soil pH plays a significant regulatory role in seed germination. This study investigated the germination characteristics of Sorghum under varying pH levels and possible ameliorative effects of seed priming using plant growth-promoting chemicals. Viable seeds of Sorghum were primed in 150ppm Indole Acetic Acid (IAA), Gibberellic Acid (GA3) and Ascorbic Acid (ASA) and that was sown in Petri dishes moistened with pH solutions at 1,3,5,7,9,11 and 13 levels. Results showed no seed germinated at extreme pH (1, 3 and 13) in the unprimed seeds, while seed germination was observed when primed with IAA, GA3 and ASA at all pH levels, except for 1 and 13. Germination time and germination percentage in the both primed and unprimed seeds were observed to show significant differences at different pH levels. Furthermore, it was observed that seeds chemoprimed with IAA showed highest average germination properties (30%) compared to other plant growth-promoting chemicals at all the assayed pH suggesting that germination characteristics of Sorghum can be improved through chemoprime even under different pH levels.

Keywords: Germination, *Sorghum bicolor* (L.), indole acetic acid, Ascorbic acid, Gibberellins, Chemopriming, Growth.

RESUMEN

El sorgo (*Sorghum bicolor* L.) Moench es un cultivo importante que se produce predominantemente en la parte norte de Nigeria. Sin embargo, el desafío de la desertificación ha afectado constantemente el pH del suelo, obstaculizando el crecimiento y el rendimiento del cultivo. El pH del suelo juega un papel regulador importante en la germinación de las semillas. Este estudio investigó las características de germinación del sorgo bajo diferentes niveles

de pH y los posibles efectos de mejora de la preparación de semillas utilizando productos químicos que promueven el crecimiento de las plantas. Semillas viables de sorgo se imprimaron en 150 ppm de ácido indol acético (IAA), ácido giberélico (GA3) y ácido ascórbico (ASA) y se sembraron en cajas de Petri humedecidas con soluciones de pH a 1,3,5,7,9,11 y 13 niveles. Los resultados mostraron que ninguna semilla germinó a pH extremos (1, 3 y 13) en las semillas sin imprimación, mientras que se observó germinación de semillas cuando se imprimaron con IAA, GA3 y ASA en todos los niveles de pH, excepto 1 y 13. Tiempo de germinación y porcentaje de germinación en Se observó que tanto las semillas preparadas como las no preparadas mostraban diferencias significativas a diferentes niveles de pH. Además, se observó que las semillas quimioprimadas con IAA mostraron propiedades de germinación promedio más altas (30%) en comparación con otros productos químicos que promueven el crecimiento de las plantas en todos los pH analizados, lo que sugiere que las características de germinación del sorgo se pueden mejorar mediante quimioprima incluso bajo diferentes niveles de pH.

Palabras clave: Germinación, Sorghum bicolor (L.), ácido indol acético, ácido ascórbico, giberelinas, quimiopriming, crecimiento.

Abbreviations

IAA= Indole Acetic Acid

GA= Gibberellic Acid

Ascorbic acid = ASA

FGP = Final Germination Percentage

FSG = First Sings of Germination

RL = Radicle Length

PL = Plumule Length

T50 = Median Time of Germination

SV = Seed Vigor

MDG = Mean Daily Germination

INTRODUCTION

Sorghum bicolor (L.) Moench. is commonly called Sorghum and is a staple food promptly available in Nigeria. Sorghum was originally a wild plant in Africa and Central Asia (Adedeji, 2020). It belongs to the family of Poaceae and its common names include millet, Sorghum, broomcorn, sweet sorghum. In the Nigerian dialects, it is referred to as ‘Okababa’ by the Yorubas, ‘dawa/jero’ by the Hausas and ‘soro’ by the Igbos (Adedeji, 2020). It is known by a variety of names globally and they are ‘milo’ or ‘milo-maize’ in the United States, ‘dura’ in Sudan, ‘great millet’ and ‘Sorghum’ in West Africa, ‘kafir corn’ in South Africa, ‘mtama’ in the eastern part of Africa and ‘jowar’ in India (Adedeji, 2020). It is the world's third most important food grain excelled by wheat and rice (Musa and Ikhajiagbe, 2021). It is the

major grain food in a great proportion of Africa, Asia and South America. It is one of the world's top cereal crops and of all the small number of grains that provide 80-85 percent of the world's food energy worldwide, only four foods namely rice, wheat, maize, and potatoes, are consumed more than sorghum. Sorghum has a profound nutritional attribute. The grain contains are starch (68-80%), protein (10- 15%), moisture (11-12%), fat (3%), fibre (2%), ash (2%) and its overall food energy is 394 calories. It ranks second to maize in total available energy among the cereal grains (Ikhajiagbe et al., 2022). Some Sorghum varieties possess high dietary fibre content but this affects the availability of some key nutrients (Knox et al., 2012).

Nigeria is the third-largest producer of Sorghum in the world coming after the United States and India (Gentili et al., 2018). Because 90% of the sorghum produced by the United States of America and India is destined for animal feed, Nigeria currently is the world's leader in food grain sorghum production (Ikhajigbe et al., 2019; Reddy, 2012). Sadly, sorghum is imported by Nigeria (Reddy, 2012). Although exportation is not carried out in Nigeria, sorghum has a greater unexploited potential than any other crop for the reasonable economic advancement of any nation (Azare et al., 2020; Rezvani and Zaefarian, 2017). Desertification as a challenge, currently impedes agricultural production in the Northern part of Nigeria, causing low agricultural output, decreased yield and even degraded arable lands for agriculture (Azare et al., 2020; Rezvani and Zaefarian, 2017). The unfriendly problem of desertification currently facing northern Nigeria, the major producers of *S. bicolor*, cannot be ignored. Between 50 % and 75 % of at least eleven states (Adamawa, Bauchi, Borno, Gombe, Jigawa, Kano, Katsina, Kebbi, Sokoto, Yobe, and Zamfara states) in Nigeria (Farooq et al., 2013) are currently affected by desertification which negatively influences environmental conditions such as light, water, oxygen, temperature and pH necessary to sustain plant growth and development (Gebeyaw, 2020). These environmental factors significantly regulate the seed germination process of crops resulting in delayed germination (Hariprasanna and Rakshit, 2016).

However, pH (the extent of alkalinity or acidity of the soil) as an abiotic factor plays a crucial role in the germination of seed and the development of plants (Hariprasanna and Rakshit, 2016). Like with all other environmental factors, plants vary in their response to soil pH. Some plants are favoured developmentally by low pH (acidic soils) and others thrive in a higher pH (basic soil) environment (El-mergawa and Abd El-Wahed, 2020). Research suggests an optimum pH range of 5.5 to 6.5 for plant survival and unarguably outliers exist which favour pH extremes (El-mergawa and Abd El-Wahed, 2020). The extent of control pH has over seed germination, crop growth and development, cannot be disdained. Plants require nutrients to grow and these nutrients are only as available or accessible as the pH allows (El-mergawa and Abd El-Wahed, 2020; Curtbew, 2020). Plant morphological traits such as height, lateral spread, biomass, flower size and even the number of flowers formed have been seen to be significantly modulated by soil pH (El-mergawa and Abd El-Wahed, 2020). For instance, for *S. bicolor* specifically, the mortality of seeds and seedlings and even diminished grain yield has been linked to its inability to thrive in acidic soils (Yazid et al., 2013). Some seeds, however, are unaffected by pH changes and this makes them a highly dominant plant and one that can occur almost everywhere irrespective of varying soil pH profiles (Hariprasanna and Rakshit, 2016). The first impact of pH is on the soil and it has a ginormous influence on the plant's ability to use soil nutrients

making soil pH a chief indisputable soil variable (Yazid et al., 2013; El-mergawa and Abd El-Wahed, 2020; Curtbew, 2020).

It is noteworthy that although soil pH has such vast control over plant growth and development, the response of crops to varying soil pH is not the same (Zhang et al., 2015). However, unvarying germination of the seed is a critical factor upon which crop establishment depends upon (Koger et al., 2004; Mundiyara et al., 2020). Although variability in germination would negatively affect seedling vigour and yield, these problems can be mitigated by seed priming (Guangwill and Tailin, 2013). Seed priming is a method that facilitates seed germination irrespective of the prevailing environmental condition (Sasi et al., 2021). Several seed priming techniques have been used under various environmental stress and they include hydropriming, osmopriming, chemical priming, halopriming, solid matrix priming, nutrient priming, Ascorbic acid (ascorbate) priming and hormonal priming Okunola and Ikuomola, 2020; Yamaguchi, 2008; Butchee et al., 2012). The unique differences between all these priming methods are the solvent in which the seeds are soaked before dehydration hence the different names (Yamaguchi, 2008; Butchee et al., 2012). Irrespective of the priming method employed, however, some other key physical and chemical components (osmotic potential, temperature, presence or absence of light, aeration and seed condition) affect the success of the priming process and determine the eventual germination rate and time, seedling vigor and subsequent plant development[22]. It is however important to note that the priming process has been employed on some crops with great success. According to Gornik and Lahuta (2017) who worked with wheat seedlings that were primed with ascorbic acid, he discovered that ascorbic acid has brought about enhanced seedling emergence, growth, yield, and the water status of the wheat under low water stress. Soomro *et al*. (2020) worked with rapeseed (*Brassica napus* L.) and they observed that ascorbic acid can significantly enhance germination, shoot length, root length, vigor index, and even promote enzyme activities under drought conditions. Seed priming with salicylic acid or jasmonic acid was demonstrated by Gornik and Lahuta (2017). to improve the growth, carbohydrate content, and low-temperature resistance of sunflower (*Helianthus annuus* L.).

Plant growth-promoting hormones are capable of healing plants of harm induced by abiotic stresses (Musa et al., 2022). Plant growth regulators play crucial functions in plants facing abiotic stress and demonstrate an appreciable ability to boost a plant's adaptation to an unstable, ever-changing environment through the modulation of the plant's growth, development and nutrient use (Humphries et al., 2018; Mamun et al., 2018; Sanchez-Moreno et al., 2018). Plant growth hormones are produced in vivo by plants in negligible concentrations to regulate physiological and morphological processes in plants necessary for their survival (Yue et al., 2021; Liu and Liu, 2011; Olorunmaiye and Olatunji, 2018). These plant growth regulators include both synthetic (salicylic acid, brassinosteroids and jasmonates) and naturally occurring growth hormones (gibberellins, abscisic acid, ethylene, auxins and cytokinins (Ebrahimi and Eslami, 2012). They adjust the plant's response to the stressed plant's physiological and molecular reaction resulting in the plant's improved chance of survival. Plant growth regulators have been implicated in the drought survival observed in *Oryza sativa* (Opik et al., 2005). The method of application is sometimes to the leaves and other times to the seed by seed priming" (Masra and Fridorich 1972; Rakshit and

Singh, 2018). Auxins (for example indole-3-acetic acid) is a plant growth hormone with multiple functions. It is unarguably a life-sustaining component of plants facing stress conditions (Sanchez-Moreno et al., 1998; Khan, 2021). Gibberellins stimulate the germination of seeds, cause leaf expansion, elongate stems, promote the development of fruits and are important when plants are facing abiotic stresses as they improve their response and profitable adaptation (Farooq et al., 2013; Daddario et al., 2017; Khan et al., 2020). Gibberellins also work with other plant growth influencers in many processes involving response to stimuli (Khan et al., 2020). Ascorbic acid commonly known as vitamin C (Daddario et al., 2017) is an antioxidant that aids the detoxification process in plants by limiting the production of harmful compounds, such as H_2O_2 and quinone, which negatively impacts plant catabolic and anabolic processes resulting in significant damage to plant cells (Liu and Liu, 2011). Ascorbic acid is the chief antioxidant present in plant cells. It supports other membrane-indentured antioxidants in its role as a cellular protectors (Okunola and Ikuomola, 2010; Daddario et al., 2017). A lot of research is currently been carried out on the benefits of Ascorbic acid in the alleviation of biotic and abiotic stress. It has been observed that vitamin C promotes the germination, growth and development of seedlings of plants such as potatoes, beans, sorghum and tomato (Colebrook et al., 2014) even in salinity and drought (Daddario et al., 2017). Therefore, the current study investigated the germination characteristics of Sorghum under varying pH levels and possible ameliorative effects of seed priming using plant growth-promoting substances.

MATERIALS AND METHODS

Experimental Area: The study was conducted in the post graduate laboratory, the Mushroom building of the department of plant biology and biotechnology, University of Benin, Benin City, Edo State. The germination experiment was carried out in the laboratory *in-vitro* from January-April 2019.

Seed collection: The seeds of *Sorghum bicolor* were obtained and subjected to viability test following Musa and Ikhajiagbe (2021).

Germination study: At first, Whatman filter paper of 42 (VWR, Atlanta, GA) was placed in different Petri dishes and soaked with 10mls of the levels of pH solution in preparation for the seeds. Then, the experiment was divided into two phases.

Phase 1 germination study (no priming): 60 viable seeds were placed in the Petri dishes at replicates of five for each pH level without priming. Growth parameters were measured for seven days of the germination study following (Gornik and Lahuta, 2017).

Phase 2 germination study (with priming): 60 viable seeds were initially primed in 10mls of 150ppm concentration of IAA, GA, Ascorbic acid (Vitamin C) and water for one hour before being introduced into the Petri dishes following (Soomro and Mazari, 2020).

Morphometric parameters

Germination percentage: The number of germinant was recorded twice daily at 15 hours intervals for phase 1 and 10 hours intervals for phase 2 before termination after seven days. The germination percentage is calculated as:

$$\text{Germination \%} = \frac{\text{Number of germinant}}{\text{Number of seed}} \text{ X } 100$$

Radicle length: The length of the radicle was measured using a meter rule in millimeters (mm) once daily for seven days.

Plumule length: The plumule length was obtained daily in millimeters (mm) for seven days.

Plant height: The plant height was recorded beginning from the second or third day of germination before termination.

Seedling vigor: The seedling vigor was determined using the formula below:

SV1 (I) = Seedling length x FGP

SV1 (II) = (Root length + Shoot length) x FGP

Biochemical parameters

The enzyme activity for four enzymes was determined as follows:

Catalase: Catalase (CAT) activity was estimated by the method described by Daddario *et al.* (2017). Phosphate buffer (pH 7.4) 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 was weighed and dissolved in 100ml of distilled water. To an unknown volume of plasma (0.5 ml), 5.0 ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5 ml of 6M H_2SO_4 and 7ml of 0.01M $KMnO_4$. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as µmoles of H_2O_2 decomposed/min/mg/protein following:

$$\text{Activity} = \frac{\text{OD/min } x\ V}{\text{M x V x L x Y}}$$

Where OD = Absorbance
L= Light path
V= Total volume of the reaction sample
M= Molar coefficient of H_2O_2 (40/m/cm)
V= Volume of sample
Y= mg protein in the sample

Superoxide dismutase: This was determined according to the methods of Ebrahimi and Eslami (2012), Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na_2CO_3, 0.2604g $NaHCO_3$ and 0.0372g of EDTA in 100 ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide. Hydrochloric acid (0.005 M) and Adrenaline solution (0.3 mM) were prepared and added with 125ml of carbonate buffer. 100ml of distilled water was mixed with 1.25 ml of carbonate buffer as the reference sample. These were mixed and absorbance read at 420 nm.

Glutathione Peroxidase: This was determined according to Khan, 2021. To an aliquot of plasma (0.2 ml), 2.5 ml of phosphate buffer, 2.5 ml of H_2O_2, 1.5 ml of distilled water and 2.5 ml of pyrogallol was added. The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Malondialdehyde: Malondehyde was determined using the thiobarbituric acid assay (Kazan 2013, Rhaman et al., 2020). A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000g for 10min. The absorbance was determined using the formula:

$$\text{MDA}\left(\frac{\text{mol}}{\text{mg}}\text{protein}\right) = \frac{\text{A x V}}{\text{M x V x Y}}\text{X } 100$$

A= Absorbance

V= Total volume of the reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

Total Sugar: 1.0ml of the sample was pipetted into a test tube. 1.0 ml of R_4 was added and boiled for 20 minutes. The tube was allowed to cool. 1ml R_3 was added and shaken vigorously to remove CO_2 and was allowed to stand for 10minutes. 10 ml of distilled water was added to the tube and was shaken well before reading spectrophotometrically at 600 nm. The absorbance value of the test sample was extrapolated from the standard curve to get the concentration (µg/ml) of sugar in the sample.

Statistical analysis: The mean of five determinations was taken using graph pad prism version five. A two-way analysis of variance was performed to determine sources of variability among the treatment used.
Germination indices were determined according to the formula below.

RESULTS

The present study investigated the germination characteristics of *Sorghum bicolor* under different pH regimes before and after chemo priming with growth-promoting chemicals such as Indole-acetic acid (IAA), gibberellic acid (GA) and Ascorbic acid (ASA). Result showed that germination occurred within the first day after initiation in the primed seeds. However, there was no germination at extreme pH of 1 and 13. Furthermore, delayed seed germinationwere observed in the unprimed seeds, but no seed germination at extreme pH of 1, 3 and 13

throughout the six days observation period (Figure 1a-d). Significant increase in germination percentage with increasing germination time was observed in the primed seeds, especially at pH 5 and 9, while delayed germination percentage with increasing germination time was observed in the unprimed seeds at all assayed pH. In the primed seeds, the highest germination percentage (97%) was attained at basic pH of 5 at day 3 in the IAA solution however, in the unprimed seed, the highest germination percentage (50%) was obtained at pH of 7 at day 6 (Figure 1d).

activities in the roots of rice (*Oryza sativa* L.) seedlings. *Plos One.* 10(8): 652-69.

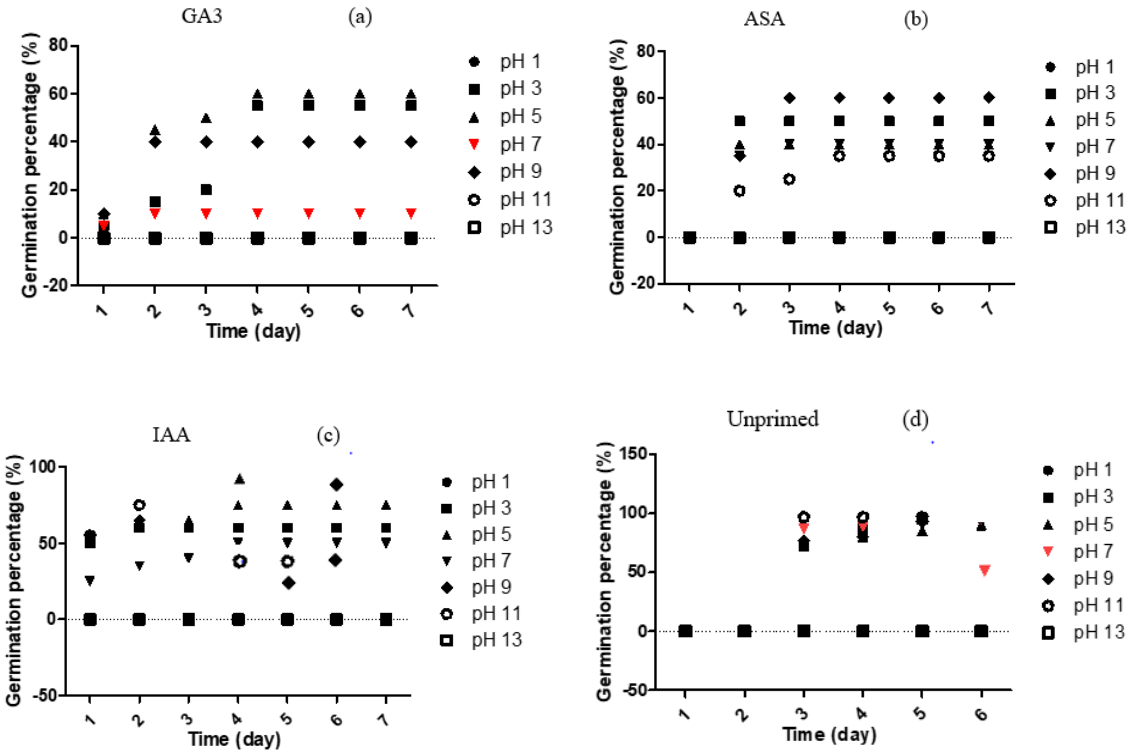

Figure 1a-d: Germination properties

Germination performance before priming was computed using germination indices that accessed germination time and germination capacity (Table 1). The time taken for the first signs of germination (FSG) to occur was 15hours at pH 3, 13 hours at pH5, and 10 hours at pH 11. However, it took up to 165 hours for *Sorghum bicolor* to complete germination (table 1). The median time of germination (T50) stood at 67 hours at pH 3, 37 hours at pH 7 and pH 11. Mean daily germination (MDG) ranged from 15.0 at pH 5 to 32.2 at pH 11 whereas germination capacity ranged from 1.5 to 1.7. Considering the variance, however, it was observed that variance was highest in an index. A two-way analysis of variance for germination indices before priming against the pH levels. The sources of variation, in this case, were pH levels and germination indices. With a mean square of 2045000 for germination indices, the latter accounted for 61.27% of total variation compared to the 6.18% variation accounted for by the pH levels.

Table 1: Germination indices before priming

Parameters	pH levels							Mean	Variance
	1	3	5	7	9	11	13		
FGP (%)	0	30	32	08	20	10	0	68.6	2204.1
FSG (hrs)	0	15	13	13	12	3.4	0	9.0	40.0
RL (cm)									6220.6
PL (cm)									7126.3
T50(hrs)	0	30	23	15	10	0	0	28.6	556.3
PH									5291.0
SVI II	0	0	0	0	0	0	0	2049.1	2329807.9
MDG	0	08	11	05	16.4	0	0	13.7	123.3

2-way ANOVA tabular results for germination indices before priming against pH levels

Source of Variation	Sum-of-square	Mean square	F	% of total variation	P-value
pH levels	4126000	687600	3.797	6.18	0.0017
Germination indices	40900000	2045000	11.29	61.27	< 0.0001
Residual	21730000	181100			

The importance of the synchronization index is a criterion for determining germination performance (table 2). It helps to show the possibility that germination would occur or not occur. When the synchronization index was significantly higher, the possibility for germination was higher compared to when the synchronization index was low which would indicate that the possibility that germination would not occur would be higher. In this study, the synchronization index was highest at pH 11 (418.22) compared to 223.57 at pH 7. Similarly, at pH 3, the synchronization index was 511.73 (Table 2). The implication is that at pH 3 and pH 5, germination was most likely to occur under the current experimental conditions compared to pH 7.

Table 3 shows the comparative effect of chemopriming with the three growth-promoting chemicals on germination indices after priming. When IAA was applied, the final germination percent (FGP) of pH7 was 12.2 compared to 10.5 and 10.1 in the GA3 and ASA respectively. Chemopriming with IAA implied a peak period of germination at 70 hours during exposure to pH 3, 73 hours in pH 5 and 44 hours in pH 7. This implication meant that as pH increased, chemopriming during enhanced germination time. The same was observed during the application of GA3 and ASA at similar pH.

Table 2: Synchronization index before priming

Day	Ni	Fi	logFi=E	FiE -1
pH 3				
1	9	9	3.1699	27.529
2	18	6	2.585	14.51
3	43	7.1667	2.8413	19.363
4	52	5.2	2.3785	11.368
5	57	71	6.1497	435.63
6	60	2.8571	1.5146	3.3274
			Total	511.73
pH5				
1	27	27	4.7549	127.38
2	43	14.333	3.8413	54.059
3	44	7.3333	2.8745	20.079
4	48	4.8	2.263	9.8626
5	51	3.4	1.7655	5.0028
6	54	2.5714	1.3626	2.5038
			Total	218.89
pH 7				
1	26	26	4.7004	121.21
2	44	14.667	3.8745	55.826
3	52	8.6667	3.1155	26.001
4	53	5.3	2.406	11.752
5	55	3.6667	1.8745	5.8731
6	57	2.7143	1.4406	2.9101
			Total	223.57
pH 9				
1	29	29	4.858	139.88
2	45	15	3.9069	57.603
3	46	7.6667	2.9386	21.529
4	48	4.8	2.263	9.8626
5	56	3.7333	1.9005	6.0951
6	59	2.8095	1.4903	3.1871
			Total	238.16
pH11				
1	54	54	5.7549	309.76
2	56	18.667	4.2224	77.818
3	58	9.6667	3.273	30.639
			Total	418.22

Table 3a: Effects of chemopriming with IAA, GA3 on germination indices after priming

	GA3							IAA						
	pH 1	pH 3	pH 5	pH 7	pH 9	pH 11	pH 13	pH 1	pH 3	pH 5	pH 7	pH 9	pH 11	pH 13
FGP	0	55.0	60.0	10.5	40.0	4	0	0	55.0	62.1	11.4	40.0	5.2	0
FSG(hrs)	0	10.0	12.3	12.3	10.0	4	0	0	12.4	10.0	13.2	12.3	9.8	0
RL(cm)														
PL(cm)														
T50(hrs)	0	60.0	40.0	20.0	20.0	2	0	0	62.1	40.0	20.0	20.0	21	0
SVI II	0	1017.5	2100.0	180.0	760.0	2	0	0	1017.5	2100.0	180.0	760.0	12.5	0
MDG	0	13.8	15.0	5.0	20.0	2	0	0	13.8	15.0	5.0	20.0	2.5	0

Table 3b: Effects of chemopriming with ASA on germination indices after priming

	ASA						
	pH 1	pH 3	pH 5	pH 7	pH 9	pH 11	pH 13
FGP	0	55.0	60.0	12.2	40.0	0	0
FSG(hrs)	0	10.0	12.3	12.3	10.0	0	0
RL(cm)							
PL(cm)							
T50(hrs)	0	60.0	40.0	20.0	20.0	0	0
SVI II	0	1017.5	2100.0	180.0	760.0	0	0
MDG	0	13.8	15.0	5.0	20.0	0	0

Germination rate index was lowest at pH 3 (18.03) when IAA was applied however, when GA3 and ASA were applied, the germination index still achieved the lowest at pH 3, even though the index were significantly lower compared to the IAA (10.1 and 10.2) respectively. Figure 2 shows the effect of pH on enzyme activity in Sorghum before chemopriming. The result showed that SOD activity in the seed was constant (93.07unit/g). However, with chemopriming using ASA, SOD activity reduced to 75.3unit/g in pH 1 and 90.85unt/g in pH 9. High SOD activity was reported in pH 13 (95.65unit/g). Chemopriming with IAA and GA did not significantly affect SOD activity as reported earlier in unprimed seeds. Catalase activity as presented in Figure 2 showed relatively uniform activity in all the treatments irrespective of the chemopriming agent. Catalase ranged from 26.02 to 29.44unit/g. Figure 2 shows the activity of glutathione peroxidase. The concentration of glutathione peroxidase in the unprimed seeds was averaged 116.53unit/g in the seeds primed with ascorbic acid, IAA and GA3.

There were minimal increases in glutathione peroxidase activity to as high as 154unit/g. The concentration of malondialdehyde (MDA) shows marked changes in MDA concentrations. In the unprimed seeds, the concentration of MDA averaged 30.5unit/g. However, for seeds primed with ASA MDA concentration at pH 1 was 53.27 compared to 32.31 at pH 7. With the application of IAA, the MDA activity was increased at pH 5(39.83) compared to 30.00 at pH 7. Generally, the results of figure 2 show enzyme activity after seeds have been chemoprimed. The activity of SOD was relatively uniform during the day.

Enzyme levels were presented as possible sources of variation (Figure 2 and Table 4). For all enzyme assayed, the result showed that the pH levels explained more than 25% of the total variation recorded in the experiment. For treatments, mean square 32.11 SOD, 0.63 for catalase, 202.9 for glutathione peroxidase and 30.5 for malonaldehyde. The implication is that variability was most likely attributed to changes in glutathione peroxidase.

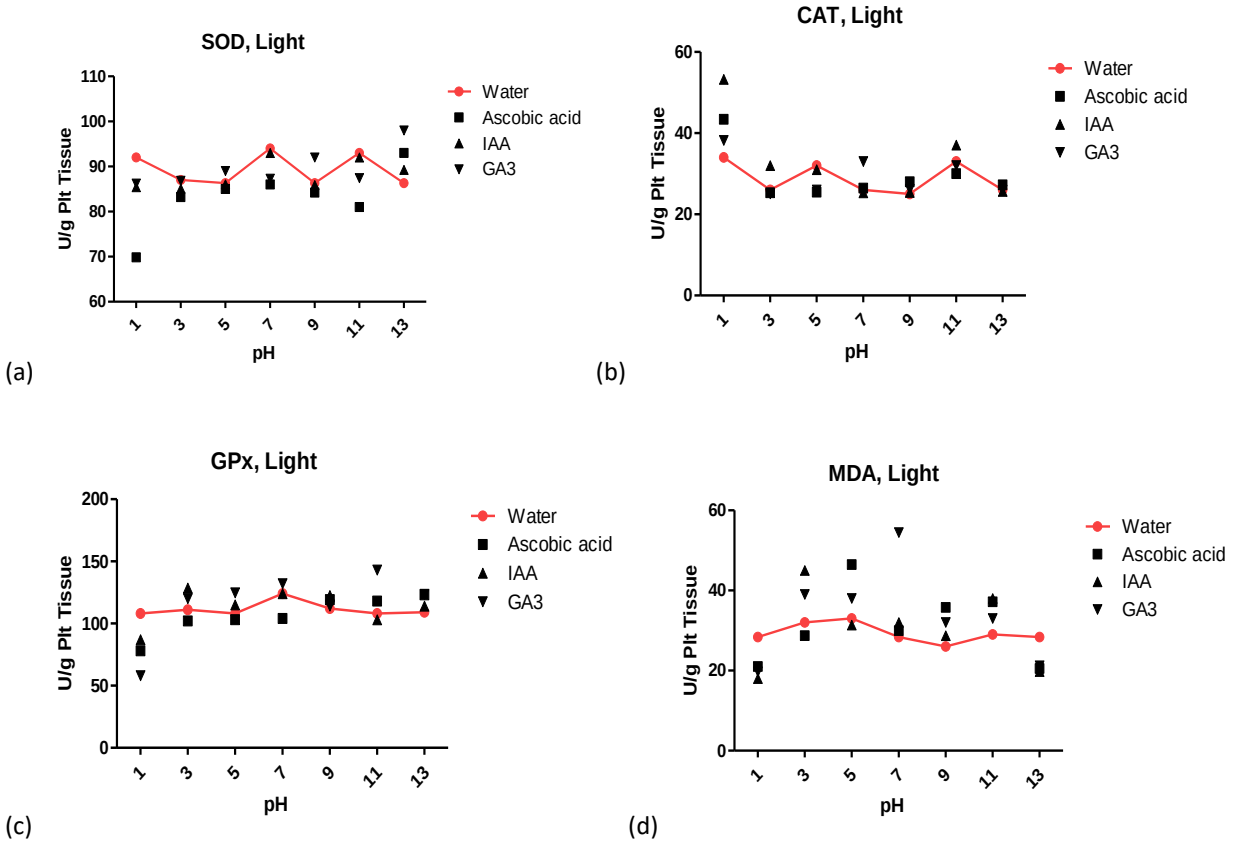

Figure 2a-d: Effects of chemopriming on enzyme activity

Table 4: Enzyme levels as sources of variation

Source of Variation	Sum-of-squares	Mean square	F	% of total variation	P-value
SOD					
Treatments	96.32	32.11	3.413	26.01	*0.040*
pH levels	104.7	17.45	1.856	28.28	0.144
Residual	169.3	9.406			
CAT					
Treatments	1.908	0.6361	2.57	17.79	0.086
pH levels	4.363	0.7271	2.938	40.68	*0.035*
Residual	4.454	0.2475			
GPx					
Treatments	608.7	202.9	0.6409	3.01	0.599
pH levels	13890	2315	7.313	68.77	*0.000*
Residual	5698	316.6			
MDA					
Treatment	91.49	30.5	0.494	4.17	0.691
pH levels	990.8	165.1	2.675	45.17	*0.049*
Residual	1111	61.73			

DISCUSSION

From the results, soil pH significantly influences the growth and development of plants during germination. The extreme level of pH affects the plant's ability to absorb the nutrient and therefore no seed germinated at pH 1 and 13. This is consistent with the report of Azare et al. (2020) that extreme soil pH can cause nutrient deficiency and thereby impairing seed germination. Butchee *et al*. (2012) studied the effect of soil pH on Sorghum and they observed that the sorghum is sensitive to soil pH changes and high plant mortality is expected at extreme pH. The high germination time and percentage witnessed in the chemoprimed seeds at different pH levels indicated the positive influence of plant growth-promoting chemicals. This study agrees with a study by Ebrahimi and Eslami (2020) who worked with *Caperonia palustris*. Ebrahimi and observed that germination was optimal within pH 5 to 9 with the introduction of biosynthesized IAA. In this study, it was observed that extremes of pH reduced seed germination response. The fast seed germination observed in the chemoprimed seeds may be linked to the growth-promoting properties of the chemical. Different bioactive substances have proved positive in reducing the cation exchange capacity of the soil, thereby influencing the soil pH and increasing chances of nutrient uptake by root nodes (Musa and Ikhajiagbe, 2021). Delayed seed germination observed in the unprimed seed could be linked to the high pH level of the soil (Opik et al., 2005). Ion toxicity and nutrient imbalances are amongst the major causes of germination impairment. The highest germination properties observed in the seeds that were primed with IAA indicated the effectivity of IAA in comparison with ASA and GA3. A previous research by El-mergawi and El-wahed (2020) have observed that despite soil acidic pH (3-4), the seed of *Nassella trichotoma* was improved after priming with various levels of IAA. Although, previous research by Jimoh and Abdullahi (2017) have explained that for a growth-promoting

chemical to improve germination parameters of seeds at elevated temperature, it must be that the growth-promoting chemical can influence the proton gradient across the seed. Since IAA was able to improve germination properties of sorghum at an extreme pH of 3, that indicated that IAA has influence the proton concentration gradient across sorghum seeds.

CONCLUSION

Sorghum is easily affected by pH, especially at extremes pH as shown in this study. Chemopriming with plant growth-promoting chemicals such as IAA, GA3 and ASA have proven effective in improving germination parameters even at high pH except for 1 and 13. This bio-product can be used in ameliorating the challenge of extreme pH especially in drought-stressed soils. Results of this research provides a unique way of neutralizing soil pH.

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