Salinity and Sodicity Induced Responses on Total Phenols, Flavonoids and Tannins Accumulation on Cowpea (*Vigna unguiculata* (L) Walp)

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Abstract

Food shortage remains a developmental hindrance in globally with about 25% of world's population suffering from continued food and nutritional insecurity especially in ASALs. Reduced arable tracts of land for cultivation, increased population and variation in non-living factors such as water and salinity are challenges facing the world. Cowpea is commonly grown on ASALs which are greatly affected by salinity. The study involved 4 cowpea varieties; K80, M66, Kunde 1 and KVU 27-1 that were subjected to both $CaCl_{2(aq)}$ and $NaCl_{(aq)}$ at levels of 0 (control), four, eight and twelve dS/m. A complete randomizeddesign in replicates of three in a greenhouse was usedat Jomo Kenyatta University of Science and Technology, JujaSubcounty, Kiambu County on October 2021-January 2022.The salts concentrations (NaCl and $CaCl_2$) were applied to the soil from time of sowing and constant levels were monitored throughout by use of an EC probe Determination of total phenols, tannins, and flavonoids content was done using a UV-VIS spectrophotometer. The obtained data wereevaluated using 2-way ANOVAat 5% significance level using SPSS (software version 21). As salinity and sodicitylevels increased, the phenols, tannins and flavonoids content also increased. Variety M66 recorded the highest total phenols, tannins and flavonoids above 8 dS/m hence it developed a defense mechanism against salt stress. Therefore, variety M66 was better adapted to high salt levels because it performed better at high saline and sodic levels. Therefore, farmers especially in marginal areas affected by salinity should adopt growing of M66 cowpea variety.

Keywords: Cowpea, Phenolics, Salinity, Sodicity, UV-VIS spectrophotometer

Introduction

Cowpea (*Vigna unguiculata* (L.)Walp)is an herbaceous crop and second most grown legume due to its leaves, pods and seeds. It's an essential crop for human and livestock consumption due to its high protein content (Padulosi and Ng, 1997). In addition, it's an essential crop widely grown in marginal regions due to its water stress tolerance (Padulosi and Ng, 1997). In Kenya, the main cowpea growing regions are Eastern and Western and the common grown varieties are Machakos 66, Kunde 1, KVU 27-1, Kitui black eye, Katumani 80 and 419 (Njonjo *et al.,* 2019). In addition, about 7.1 M tonnes of cowpea seeds are produced yearly. Cowpea production globally is approximately 3.1 million ha and 1.1 M tonnes grain production annually (Singh *et al.,* 2020).

Kenya occupies 582,646 sq. km land area an approximate of 60 million ha in which 80% is covered by the ASALs which are greatly affected by salinity (Sijali and Okumu, 2002). Salinity commonly occurs due to weathering of mineral rocks. Also limited irrigation causes accumulation of salts especially in marginal areas. The main mineral elements responsible for salinity include; Potassium, Sodium, Calcium, Magnesium, Chlorine, carbonates and Sulphateions (Li *et al*., 2014). Soils with above 4 dS/m salt concentrations are said to be saline and this decreases the productivity of the crops. Plants are grouped as halophytes or glycophytes based on their salinity tolerance ability (Gupta and Huang, 2014). In marginal regions, there is limited leaching of soluble salts hence salts accumulate over time resulting to saline soils which negatively impact the production of cowpea (Nkaa *et al.,* 2014).

Plants response to physiological stress involves alteration of biochemical synthesis pathways such as production of metabolites (Selvam *et al.,* 2013). Salinity stressed plants have increased release of secondary metabolites hence degradation of cell membrane which results to death of the whole cell (Hernández *et al.,* 2014). Kusvuran, (2012) stated that many researchers have reported deficiency of water in older leaves and carbohydrates in young leaves due to long term salinity stress. Under stress conditions, resistance of salt therefore depends on the plant's ability to develop adaptive strategies such as synthesis of secondary metabolites (Ors and Suarez, 2016).

Increased levels of secondary metabolites lower agricultural produce furthermore they help to counteract physiological stresses (Navarro *et al.,* 2006). Excess irrigation water leaches out dissolved salts which reduces accumulation of salts on the top soil surfaces (Li *et al.,* 2014). Due to the continued water shortage and water resources, washing out salts from soil surface through drainage is not a better option to solve salinity problems (Higbie *et al.,* 2010). To control salts stress effects on crop performance, the best alternative is to grow salt-tolerant crops (Higbie *et al.,* 2010).Therefore through this study, the biochemical responses, adaptation and salinity tolerance of commonly grown cowpea varieties will be evaluated.

Materials and Methods

Study area

The study was carried out in a greenhouse at Jomo Kenyatta University of Science and Technology, Juja Subcounty, Kiambu County on October 2021-January 2022. The greenhouse temperature ranged between 15 and 33 ^{o}C with a relative humidity ranging from 40 to 80%.

Procurement and selection of cowpea seed

Four varieties of grain cowpea: KVU 27-1, Machakos 66, Katumani 80 and Kunde 1 were procured from the KALRO seed unit (Machakos). Selection of seeds was conducted according to International Seed Testing Association rules (ISTA, 2009).

Research design and application of treatments

The four varieties of cowpea were assessed under 4 salinity concentrations; zero, four, eight and twelve dS/m of NaCl and $CaCl_2$. The experimental design was complete randomized design with 3 replications. An Electrical Conductivity probe (FieldScout Direct Soil EC Meter model 2266FS) was used to maintain the salinity levels (Malarde *et al.,* 2008). The salts concentrations (NaCl and $CaCl_2$) were applied to the soil from time of sowing and constant levels were monitored throughout by use of an EC probe.

Crop performance and data collection

Inert sand mixed with forest soils at a ratio of 1:3 respectively was put in6 kg plastic pots. Two seeds from the 4 varieties were planted in each pot in replicates of three at 4-6 cm deep and thinning took place on the 3rd week. Three young leaves were harvested on the 5th week and total phenols, flavonoids and tannins tests were carried out at JKUAT Food Science laboratories.

Determination of total phenolic content
Four young leaves were oven-dried (80 ^{o} C) for 30 minutes then weighed and crushed with 25 mL of distilled H_20 using a mortar and pestle then a magnetic stirrer was used to stir the resultant paste for 1 hour at 70 ◦ C. One gram of the leaf extract was placed to conical flask followed by 2 mLFolin-Ciocalteu reagent solution then 5mL of Na_2CO_3 solution. Finally, resultant solution was incubated with intermittent shaking for colour formation for about 30 minutes. The colour absorbance was read at 765 nm (UV-VIS spectrophotometer (Shimadzu- uv-1601; India) (Siddhuraju and Becker, 2017).Then a standard curve prepared with gallic acid.

Determination of flavonoids content
Four young leaves were dried at room temperature then 1.0 g of dry samples were crushed in 20 mL methanol (80%) and ultrasonically extracted. The resultant solution was centrifuged for about 30 minutes at 4000 RPM(Dash*et al.,* 2012). 3 mL of distilled water, 0.3 mL of 5% $NaNO_2$ and 3 mL of 1% $AlCl_3$ solution were added to 1 mL of the sample in a flask followed by 2 mL of NaOH (1 M) solution. Rutin made standard curve was used and total flavonoid content of samples was obtained in mg/g at an absorbance of 510 nm (UV-VIS spectrophotometer (Shimadzu-uv-1601; India).

Tannin extraction and determination
This was done according to (Yang *et al.,* 2009), using Vanillin-Hydrochloric Acid. Four young leaves were oven-dried (80 ^{o} C) for 30 minutes then ground. 0.4 g of the extract was placed into 10 mL Erlenmeyer flasks. Then 20 mL of 4 % HCl in methanol was placed to the flasks. The mixture was agitated and extract was decanted. Then centrifugation of the extracts was done at 4500 rpm for 8 minutes then transferred to 50 mL flasks. A catechin standard curve was prepared using methanol. Then 10 mL of freshly prepared vanillin-HCl reagent added. The standard was treated and 10 blanks prepared in a similar way and colour absorbance read at 765 nm (UV-VIS spectrophotometer (Shimadzu- uv-1601; India) (Siddhuraju and Becker, 2017).

Data Analysis
Two-way ANOVA was used in determining the variation in compounds among four cowpea varieties at 5 % significance level. The factors were; four cowpea varieties and 4 concentrations; the two salts were analysed independently to evaluate the variation in response among the cowpea varieties and also concentrations. Boniferroni test was used for comparing the means and SPSS (software version 21) was used for analyses.

Results
Effect of salinity and sodicity levels on total phenolic content
The result of the study indicated that an increase in salinity caused an increase in total phenolic content (Table 1). A significant interaction between the varieties and salt levels in the total phenolic content was noted. Under NaCl, variety M66 recorded the highest total phenols (191.6 mg/g) at 12 dS/m. A significant difference ($p \leq 0.05$) in total phenolic content in all treatments for all the varieties was observed. Under $CaCl_2$, there was significant difference ($p \leq 0.05$) in total phenolics for all treatments in all the varieties.

Table 1: Effect of soil salinity and sodicity (dS/m) on total phenolic contents (mg/g) in four cowpea varieties.

Sodium Chloride					
	Sodicity	K80	M66	KUNDE 1	KVU 27-1
	0	146.9±0.1^{d}	130.5±0.1^{c}	130.5±0.1^{a}	140.7±0.0^{b}
	4	146.9±0.1^{d}	141.5±0.1^{e}	141.5±0.1^{c}	150.4±0.1^{f}
	8	170.9±0.2^{i}	181.3±0.1^{k}	156.3±0.2^{g}	178.4±0.2^{l}
	12	174.3±0.1^{j}	191.6±0.1^{l}	160.4±0.2^{h}	180.7±0.1^{m}
p variety		<0.0001	<.0001	<0.0001	<0.0001
p treatments		<0.0001	<.0001	<0.0001	<0.0001
p variety:treatments		<0.0001	<.0001	<0.0001	<0.0001
Calcium Chloride salinity					
	0	131.8±0.0^{a}	141.5±0.1^{d}	146.5±0.2^{f}	134.5±0.1^{b}
	4	139.5±0.2^{c}	156.1±0.1^{k}	152.3±0.2^{h}	145.0±0.2^{e}
	8	141.8±0.1^{d}	160.3±0.0^{l}	154.5±0.1^{j}	145.5±0.2^{e}
	12	147.3±0.0^{f}	164.5±0.1^{m}	153.6±0.2^{i}	151.3±0.1^{g}
p-value(variety)		<0.0001	<0.0001	<0.0001	<0.0001
p-value(treatments)		<0.0001	<0.0001	<0.0001	<0.0001
p-value (variety:treatments)		<0.0001	<0.0001	<0.0001	<0.0001

Mean values in same column or row denoted by similar letters are not significantly different at $p < 0.05$.

Effect of salinity and sodicity levels on total flavonoids content

Comparison of means showed that an increase in flavonoids content as salinity and sodicity levels increased in all the four varieties (Table 2). Under NaCl, variety M66 recorded significant differences ($p \leq 0.05$) in the total flavonoids as sodicity levels increased although it recorded the highest total flavonoids (517.6 mg/g) at 12 dS/m. Under $CaCl_2$, all varieties were significantly affected as salinity levels increased.

Effect of salinity and sodicity levels on total tannins content

As salinity increased, tannins content increased for the four varieties (Table 3). A significant interaction between the varieties and salt levels in total tannins content was noted. Under NaCl, variety M66 recorded the highest tannins composition (111.7 mg/g) while Kunde 1 had the lowest tannins composition (73.6 mg/g). The total tannins for varieties M66, Kunde 1 and KVU 27-1 were significantly different at $p \leq 0.05$ at all treatments under $CaCl_2$.

Table 2: Effect of soil salinity and sodicity (dS/m) on total flavonoids contents (mg/g) in four cowpea varieties

Sodium Chloride					
	Sodicity	K80	M66	KUNDE 1	KVU 27-1
	0	399.2 ± 0.4^{c}	390.4 ± 0.2^{b}	389.5 ± 0.2^{a}	396.6 ± 0.1^{a}
	4	433.6 ± 0.2^{g}	461.4 ± 0.2^{h}	407.6 ± 0.2^{d}	442.3 ± 0.2^{k}
	8	445.7 ± 0.1^{i}	448.6 ± 0.2^{l}	411.7 ± 0.2^{e}	471.6 ± 0.1^{m}
	12	453.5 ± 0.1^{j}	517.6 ± 0.2^{n}	422.5 ± 0.2^{f}	493.3 ± 0.2^{o}
p-value(variety)		<0.0001	<0.0001	<0.0001	<0.0001
p-value(treatments)		<0.0001	<0.0001	<0.0001	<0.0001
p-value(variety:treatments)		<0.0001	<0.0001	<0.0001	<0.0001
Calcium Chloridesalinity					
	0	390.7 ± 0.2^{a}	391.8 ± 0.0^{b}	395.4 ± 0.2^{c}	390.3 ± 0.2^{a}
	4	397.6 ± 0.1^{d}	437.4 ± 0.0^{k}	415.6 ± 0.1^{f}	403.5 ± 0.2^{e}
	8	417.4 ± 0.2^{g}	448.5 ± 0.2^{m}	437.2 ± 0.1^{k}	424.7 ± 0.1^{h}
	12	427.5 ± 0.2^{i}	451.4 ± 0.2^{n}	444.5 ± 0.2^{l}	430.4 ± 0.2^{j}
p-value(variety)		<0.0001	<0.0001	<0.0001	<0.0001
p-value(treatments)		<0.0001	<0.0001	<0.0001	<0.0001
p-value(variety:treatments)		<0.0001	<0.0001	<0.0001	<0.0001

Mean values in same column or row denoted by similar letters are not significantly different at p<0.05.

Table 3: Effect of soil salinity and sodicity (dS/m) on total tannins contents (mg/g) in four cowpea varieties.

Sodium chloride					
	Sodicity	K80	M66	KUNDE 1	KVU 27-1
	0	0.00 ± 0.0^{d}	15.4 ± 0.2^{f}	0.00 ± 0.0^{b}	15.6 ± 0.2^{a}
	4	78.0 ± 0.1^{g}	81.3 ± 0.0^{g}	57.5 ± 0.1^{b}	79.4 ± 0.2^{h}
	8	82.1 ± 0.0^{h}	94.1 ± 0.3^{i}	64.0 ± 0.1^{c}	85.7 ± 0.1^{j}
	12	87.4 ± 0.1^{i}	111.5 ± 0.2^{j}	73.6 ± 0.2^{e}	93.1 ± 0.0^{k}
p-value(variety)		<0.0001	<.0001	<0.0001	<0.0001
p-value(treatments)		<0.0001	<.0001	<0.0001	<0.0001
p-value(variety:treatments)		<0.0001	<.0001	<0.0001	<0.0001
Calcium Chloridesalinity					
	0	0.0 ± 0.0^{c}	14.3 ± 0.1^{b}	0.0 ± 0.0^{ij}	11.3 ± 0.0^{a}
	4	29.3 ± 0.0^{d}	31.3 ± 0.0^{f}	31.0 ± 0.0^{ef}	30.2 ± 0.2^{e}
	8	31.9 ± 0.4^{fg}	42.9 ± 0.0^{l}	41.5 ± 0.1^{k}	33.1 ± 0.0^{hi}
	12	32.3 ± 0.1^{gh}	47.3 ± 0.1^{m}	42.5 ± 0.1^{l}	34.5 ± 0.0^{j}
p-value(variety)		<0.0001	<0.0001	<0.0001	<0.0001
p-value(treatments)		<0.0001	<0.0001	<0.0001	<0.0001
p-value(variety:treatments)		<0.0001	<0.0001	<0.0001	<0.0001

Mean values in same column or row denoted by similar letters are not significantly different at p< 0.05.

Discussion

An increase in synthesis of specialized metabolites in the life span of a plant has been noted due to stressful factors such as salinity (Rouphael *et al*., 2018). The total phenolic content increased as salt levels rose in the four varieties. A significant variation in the total phenols was noted at salinity levels above 8 dS/m. The synthesis phenolic compounds may be attributed to as a defense mechanism against high salt levels. According to (Dominguez-Perles *et al.,* 2011), plants counteract the salt stress effect to maintain a stable biochemical pathway which involves synthesis of phenolics. Variety M66 recorded significantly higher total phenols at each salt level. This implies that it can defend itself against salt stress hence being able to survive under salt stress. According to Pitman and Läuchli, (2002), high salinity levels severely influence the phenolic compounds composition in plant which is a defense mechanism common among the saline tolerant varieties hence improved metabolic processes.

The flavonoids composition also increased as salt levels increased for the four varieties. A significant variation in the total flavonoids above 8 dS/m was observed. The synthesis flavonoids may be attributed to as a defense mechanism against high salt levels (Hussain *et al.,* 2018). Abiotic stress causes synthesis of flavonoids which accumulate on plant tissues (Neffati *et al.,* 2011). Variety Kunde 1 had the lowest flavonoids at high salts levels which implied that it could not defend itself against high salts levels. According to Atia *et al.,* (2011), low flavonoids accumulation in plant tissues causes high decomposition rates and toxicity in plant tissues hence poor performance and salinity tolerant. Flavonoids synthesis provides a defense mechanism against damage of metabolic process to physiological stresses. According to Atia *et al.,* (2011), plants with root nodules have ability to import assimilates into the apoplast, therefore its ability to tolerance salinity is associated with its ability to protect photosynthetic processes, high metabolism and flavonoids synthesis under elevated salinity levels (Salah *et al.,* 2011).

From the results obtained, as salinity and sodicity levels increased, a significant increase in tannins composition was also noted. A significant variation was observed at salinity levels above 8 dS/m for the four varieties. The increased synthesis of tannins may be attributed to defense mechanism and antioxidant activities against high levels of salts. According to Gülcü *et al.,* 2011, tannins lower the crop quality in elevated levels by affecting the nutrient cycling through complexing proteins and inhibiting enzyme activities. Tannins synthesis in M66 was significantly higher at high salt levels. This shows that M66 is a more salt-tolerant cowpea variety. Increased phytochemical content such as tannins in plants poses a good environmental adaptation and tolerance which improves the crop yields (Rouphael *et al.,* 2018).

Conclusion and Recommendations

From the results, it's clear that the total phenols, tannins and flavonoids in cowpea increased with increase in salinity and sodicity levels. The more tolerant variety (M66) produced morephenolics, tannins and flavonoids hence able to survive under high salt stress conditions. Therefore, farmers in areas whose soils are saline should adopt growing M66 cowpea variety which showed tolerance to higher levels of salts hence would be more beneficial. More also, salinity levels below 8 ds/m are appropriate for growth of cowpea hence avoidance of losses in yield and its components Finally, further studies could also be done on M66 through future breeding programs as a source of salt tolerant genes.

Disclosure
The authors declare that this is an original article which has not been published before and is not under consideration in another journal.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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